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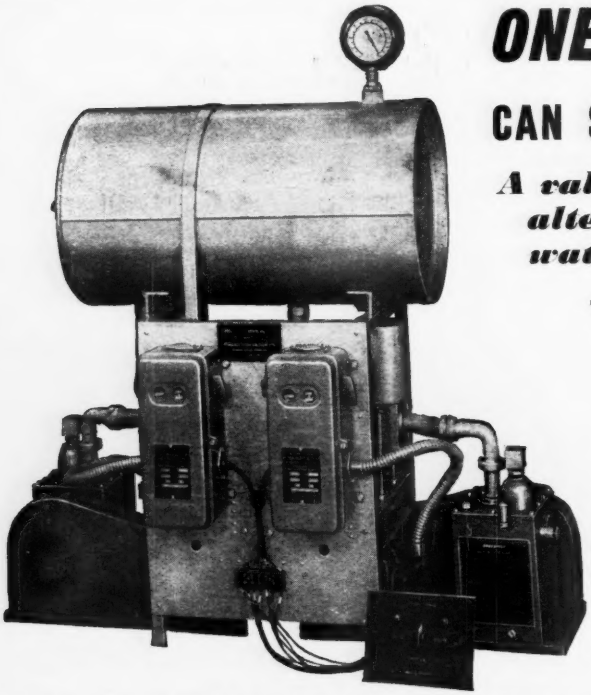

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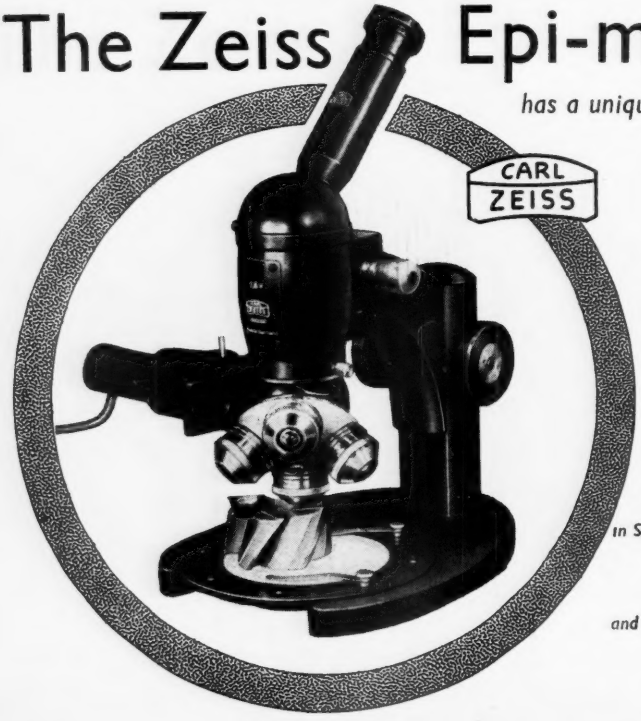

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IMPACT OF SCIENCE AND TECHNOLOGY ON HUMANISM

IN an outstanding oration, "A Balance Sheet for Science and Technology in Europe", delivered before the Conference of European University Rectors and Vice-Chancellors at Dijon on September 9, Sir Eric Ashby examined the proposition that twice in history European civilization has been one fabric, and twice in history the fabric has been torn; science and invention have contributed to its destruction, but in science and invention lies our hope for its repair. The early fabric of European society which the barbarian invasions destroyed was, Sir Eric pointed out, one in which the constant diffusion of ideas produced "a singularly uniform and cosmopolitan civilization throughout the Roman world from which the local and provincial spirit was strikingly absent". Under the impact of these invasions the common language decayed, traffic between the centres of culture diminished, the channels of communication between university and university, city and city, became overgrown and stagnant and Europeans became provincial.

When the traffic of ideas again revived, it was not, however, a traffic between institutions or corporations but a traffic between individual scholars, and the directing influence was no longer theology or the Church, but the New Philosophy and the phenomena of Nature. In the seventeenth and eighteenth centuries, science became one of the threads which held together the fabric of European civilization. A traffic which began between individuals became consolidated in the interchange of publications between scientific societies and academies, and in the nineteenth century science became established in the universities; and the universities began to regain their lost prestige in European life and have once again become centres of high intellectual energy in Europe. Sir Eric rightly stressed the importance of their intercommunications, and especially of those channels of communication which lie in science.

Turning to consider the balance sheet of science in Europe, Sir Eric rejected the view that science is the prime cause of the fear which over-shadowed contemporary life, that science can be held responsible for destroying craftsmanship and cheapening taste, or that mass production and mass communication have made Europe decadent and vulgar. Mankind, he reminded his audience, has always lived in fear, and it is only the sophisticated who suffer from a sense of doom. Again, while science and invention have changed standards for the rank and file of Europeans, the new standards have practically all been improvements: only the sophisticated show a sense of frustration, and that because, while millions are happier through the achievements of technology, they themselves are no longer able to believe in progress.

From such considerations Sir Eric argued that we should not measure the impact of science and invention on Europe by the sophisticated standards of university professors. For the common citizen of Europe, science and invention have neither increased the level of fear nor led to a decay in standards. In drawing up a balance sheet for science in Europe, fear and decadence should not be included on the debit side.

Nevertheless, there are ambivalent effects. For example, improvements in communication have proved of dubious benefit. Indeed, air travel has actually weakened some traditional bonds of understanding: the aeroplane has debased the art of diplomacy and in some ways has initiated the cohesion of Europe. Similarly, in spite of much on the credit side, the effects of mass media have a heavy debit too.

On the credit side of the balance sheet for science in Europe, Sir Eric put first the new interdependence which unites the scientific laboratories of Europe as closely as the cathedrals and priories and abbeys of medieval Europe were united. The clear and undisputed benefit which science confers on Europe, and indeed on the whole literate world, is that it deals in universals. Secondly he put a common faith in science which is shared by thousands of Europeans, and which could become a foundation from which they can explore and understand their differences. The scientists, moreover, are united not only by a common faith but also by obligations to a common faith. Thirdly, Sir Eric put the destruction by the scientific revolution of what he called the tyranny of dogmatic truth, and its replacement by a formula for tolerating error in others. Truth is now tentative, not final and revealed, and it is an integral part of the scientific tradition that this formula does not permit a man to tolerate what he has come to see are errors in his own thinking; it permits him only to tolerate what he believes to be errors in the thinking of his colleagues.

There is indeed substance in Sir Eric Ashby's contention that if there is to be cohesion in Europe, nothing is more necessary than a formula for tolerating error; nor does he exaggerate when he maintains that the scientists and other scholars of Europe comprise a coherent group preserving their national differences but sharing common beliefs and aspirations. It is a precious asset, too, that universities, since they are the chief centres of scholarship in Europe, are united by science into one pan-European community. Nevertheless, the hope for Europe depends upon cohesion among whole populations of Europeans, not solely among a handful of university teachers, and Sir Eric did well to remind us that, in spite of modern universal education, the common man to-day is less in touch with what is best and most significant in European civilization than his ancestors were five centuries ago.

If science is to make its potential contribution to European cohesion, the common man must be helped to understand something of the objectivity, the humility and the tentativeness which characterize a scientist when he is thinking about science. This we have, in spite of the mass media, so far conspicuously failed to do, and in the concluding passage of his oration Sir Eric Ashby made several practical suggestions regarding the interpretation of science. Frequently, he believes, such failure is the fault not of the mass media as such but of the interpretation itself, which has been ill-designed for the purpose to be served; and like others he directed attention to the dangers attendant on the immense prestige of science even when the work of the scientist is not understood. It must be recognized that science has its failures as well as its successes, that it too has its limitations, and as a small practical contribution to the interpretation of the values of science to the ordinary man, Sir Eric made the sound suggestion that much more could be made of the essential part played by technicians, craftsmen and instrument makers.

Here undoubtedly there is a link between science and the non-scientist, and an opportunity to enlist the interest of the man who works with his hands and to convince him that he is not just a spectator, but an essential actor, in the modern scientific age. Quite apart, moreover, from that possibility, greater attention to the place and work of the technician and craftsman could have most valuable results in other ways, for the consequent fuller understanding could in turn help the more effective use of scientists and technologists themselves and assist in maintaining a better balance in trained man-power. Such considerations should not be overlooked by the universities, and it is to them that Sir Eric Ashby turns in particular for more effective interpretation of science to the common man. This, he suggests, is a task for which they should assume greater responsibility and the execution of which they should study intensively. He believes that they have here a unique opportunity for influencing the history of Europe, such as has not been theirs since the fourteenth century. Like Sir Solly Zuckerman, he emphasized the embarrassing dependence of the decisions of governments on scientists and technologists, many of whom are based upon universities. If the universities were able to interpret to the common man the invisible forces which are thus shaping mankind, he believed they could make a notable contribution to the restoration of the fabric of Europe.

Sir Eric Ashby did not attempt to indicate further how this task should be undertaken, but it clearly enhances both the regional responsibilities of the universities and puts their extra-mural work in a fresh perspective. There are implications, too, for the use and development of the mass media which need urgent exploration; but Sir Eric was careful to disclaim any suggestion that science alone would solve Europe's problems. Science can help to spread goodwill and tolerance, but in so doing it is only reinforcing an older and deeper faith: the faith of men in mankind.

It will be noted that Sir Eric did not suggest that this task of interpreting science so that it can make its full contribution to international cohesion is solely the responsibility of the universities, although he stressed the uniqueness of their part. Other institutions have also a part to play, and some of the opportunities were clearly present in Dr. A. T. Waterman's mind when he addressed a Conference for Executives in Federal Science Agencies at Williamsburg on September 14 on science and international affairs. Dr. Waterman was primarily concerned in this address with the manner in which international scientific activities are organized, but while he stressed the dependence of national security and welfare upon science, he also emphasized the way in which science itself grows and develops without reference to national boundaries.

Much of the interest of Dr. Waterman's address lies in his account of what the National Science Foundation, of which he is director, is doing to foster international co-operation in science and the interchange of scientific information; but, like Sir Eric Ashby, he referred also to the important part which the scientific societies and learned bodies themselves have to play. Dr. Waterman, reiterating Dr. L. V. Berkner's comment that such co-operation is still very young and is best conducted by scientists themselves, pointed out the local character of certain kinds of research; and since they arise in a particular place in a particular way, the results cannot be applied universally. Dr. Waterman, however, was pleading for adequate support for such research from other regions, quoting tropical diseases and antarctic weather conditions, whereas Sir Eric referred to the ecology of alpine vegetation and was illustrating the way in which a narrow definition of the humanities could draw a distinction harmful to scholarship.

Nevertheless, Dr. Waterman, like Sir Eric Ashby, directs attention to the need for greater knowledge and better understanding both on the part of scientists and of those who serve their country abroad. Laymen in all fields should be much more literate with respect to science than in the past, and Dr. Waterman urged that universities should be encouraged to include special courses in science for non-scientists. Indeed, much more is already being done in that field in the United States than would appear from Dr. Waterman's remarks, and both the National Science Foundation and the Science Adviser to the Secretary of State have been active in that field.

Even closer to the trend of Sir Eric Ashby's oration is the fifth Dunn Memorial Lecture delivered before the Society of Chemical Industry by Sir Alexander Fleck last May. Opening with a tribute to Dr. J. T. Dunn as scientist and humanist, whose life worthily reflected that great concept of humanism which is one of the glories of our Roman heritage, Sir Alexander expounded his view of science and humanism as a background to citizenship. Like Sir Eric Ashby, he protested against a narrow definition or conception of humanism, and urged that the humanities must include the great peaks of imagination of the human mind, even if, like the

"Principia" and "The Origin of Species", their subject-matter belonged to science. While science itself is concerned mainly with fields external to man, Sir Alexander rejected the idea that scientists tend to be both lacking in humanity and incapable of appreciating the humanities.

Sir Alexander quoted Einstein's assertion, "Concern with man and his fate must always be the chief aim of scientists"; but although he obviously shares the same comprehensive view of science as Sir Eric Ashby, he did not elaborate on how such a scholarly and objective study of Nature, including man and his works, could become a new cement of civilization. He sought rather to show how the relevance of science and humanism to citizenship could find expression in practical ways. The scientist, he observed, cannot contract out of an interest in human affairs. Neither scientist nor humanist, if versed only in his narrow specialized subject, could expect to see the significance of his work in the widest human context.

Such practical contributions as Sir Alexander described clearly have their own indirect contribution to make to the interpretation of science to the ordinary citizen in the sense indicated by Sir Eric Ashby. Moreover, in its turn the task of interpretation, and perhaps especially that part of it to which Sir Eric summons the universities, has its own practical implications in another way. Both Lord Weeks, speaking as 'special visitor' at the Imperial College of Science and Technology on October 22, and Mr. H. Wilmot, in his fifth Elborne Memorial Lecture to the British Institute of Management on October 30, have recently spoken of the demands made to-day for leadership, and the way in which the universities can help to meet that need.

Lord Weeks, for example, referred to the intangible qualities of mind and personality required to make a man acceptable as a leader, to the call for high intellectual capacity and wide vision as well as for the capacity to understand the latest advances and discoveries of science and technology. Mr. Wilmot formulated even more precisely the nature of the demands made to-day upon those who carry the responsibility of positions of high authority in industry and commerce, and indicated how the universities would increasingly be expected to help provide a continuous supply of those capable ultimately of filling such office. More specifically, Lord Weeks indicated the implications for our educational system of a demand for leaders with such breadth of learning without incurring a debasement of standards.

Lord Weeks was confident that the Imperial College and other universities and colleges of Great Britain can meet this demand; but clearly this is a task to which further thought must be given. Our conceptions of both science and humanism must be sound, and every effort must be made to close that dangerous cultural gap of which Sir Charles Snow has given emphatic warning. Moreover, in the measure in which the universities are the better equipped to provide leaders of imagination, of vision and character as well as of intellectual ability and technical skill, they may find it the easier to discharge the responsibility for the interpretation of science to the common man. However that function may be best fulfilled by the universities, whether directly or indirectly, whatever new techniques may be applied, however limited or generous a view is taken of extramural or regional responsibility, a university can scarcely address itself to the task at all unless, within its own walls, the gap is closed, and science has demonstrated its cohesive power and is already binding together a community of scientists and other scholars.

MAN-MADE DRUGS

May's Chemistry of Synthetic Drugs

Revised and rewritten by Dr. G. Malcolm Dyson, in consultation with Dr. Percy May. Fifth edition. Pp. vii + 678. (London: Longmans, Green and Co., Ltd., 1959.) 110*s*. net.

DURING the twenty years that have passed since the last edition of this book our knowledge of synthetic drugs has increased out of all recognition, particularly in the fields of the antibiotics, antimalarials and sulphonamides, where work was stimulated by their military importance.

This has necessitated the re-writing of the whole book. In doing so, the authors have rightly decided to classify the drugs according to their biological activity rather than by their chemical structure.

Though the high standard set by the sensible and well-balanced views on structure and activity relationships expressed in the early chapters is maintained throughout, it is a pity that the reader is not brought quite up to date. The historical background to the various sections is excellent and useful, but some of the more recent work is not included. This point is well brought out by a study of the references. In "Metabolism of Drugs" (Chapter 2) the latest reference is Dittmer and du Vigneaud (1947), while, with the remarkable exception of Borrisov (1994) (*sic*), the most recent reference cited in the discussions on structure and activity is dated 1945. Seventeen of the forty references at the end of this latter chapter were published in the past century.

This fact in no way detracts from the authors' treatment of the subject but serves to illustrate the need for a book of this kind to be written by a panel of authors, each able to give a really up-to-date account of his own particular field. Many of the omissions are possibly due to the same cause. No mention is made of methyl pentynol in the chapter "Sedatives and Hypnotics", or of the oral hypoglycæmic agents—for example, sulphonyl ureas, or the oral diuretic chlorothiazide.

The section dealing with penicillin contains some serious errors. On p. 611, the acid hydrolysis of penicillin is said to produce penicillic acid, instead of penillic acid. Penicillic acid is correctly described elsewhere in the same chapter. In the description of the synthesis of penicillin V on p. 615 the yield for the cyclization of the β-lactam ring is quoted as 75 per cent. The actual figure should be 10–12 per cent. 75 per cent is the yield for the removal of the *t*-butyl group in the previous stage. No mention is made of the important property of acid-stability exhibited by penicillin V. That "it is not known

whether valine, cysteine and phenylacetic acid are biochemical precursors of penicillin" is not true in the light of the work by Dr. Arnstein and his collaborators.

In spite of these minor criticisms, the book is well laid out and well printed. Workers in the field of synthetic drugs will sympathize with the authors' problem of how to deal with the many trivial names of a single drug. Though perhaps not bringing the reader quite as up to date with the subject as he might wish, this book will, nevertheless, prove invaluable as a reference work to those interested in the chemistry and activity of synthetic drugs.

F. R. Batchelor

MICROBES ON THE MOVE

Biochemical Engineering

Unit Processes in Fermentation. Edited by Dr. R. Steel. Pp. 328. (London: Heywood and Co., Ltd., 1958.) 50*s*. net.

Progress in Industrial Microbiology

Vol. 1. Edited by Dr. D. J. D. Hockenhull. Pp. viii+248. (London: Heywood and Co., Ltd., 1959.) 50*s*. net.

SINCE the advent of the commercial production of antibiotics in the 1940's, the application of microorganisms to industrial purposes has been given a new stimulus, which has more than compensated for the declining importance of fermentation processes for the production of such materials as alcohol, acetone and butanol under the impact of the petrochemicals industry.

These two publications represent the first attempt in Great Britain to collect within a reasonable compass some of the vast store of information in this field. As such they will be welcomed by all whose pre-occupation with a particular aspect limits their view of the horizon.

Steel's book is based on a series of lectures given in Manchester in 1957 by a panel of speakers, who, for the most part, have considerable experience in research or in the industrial applications of microbiology. Its scope is limited essentially to the fermentation industries, which cover only part of the activities of the biochemical or biological engineer. This is clearly seen by comparing the coverage of this volume with the topics mentioned in the introductory chapter on "The Scope of Biochemical Engineering". Only one chapter is contributed by chemical engineers practising in this field, and this is concerned largely with mechanical engineering design. Its title is, therefore, a misnomer. Nevertheless, it provides an introduction to a field in which chemical engineers are likely to be increasingly involved. Indeed, its very limitations should act as a stimulus to a more forthright chemical engineering approach. The book will also serve to give those entering this field some notion of the problems and approach of the biochemist and biologist, and of the multifarious activities of the ubiquitous microbe.

The second book, from the same publisher, is the first volume in what promises to be a valuable series. The topics chosen represent a good balance between the more academic approach of the 'pure' microbiologist and biochemist on one hand, and the needs of microbiological technology on the other. The balance is maintained in the chapters on the tetracyclines, microbial fat and riboflavin synthesis. The editor himself contributes an authoritative review of the influence of the constituents of the medium on the biosynthesis of penicillin, and pays a well-deserved tribute to Dr. Bisset for his "lucid and beautifully illustrated article" on the saprophytic Actinomycetes. Finally, an article on microbiological assay not only surveys the common laboratory techniques but also includes a section on statistical analysis, showing clearly the influence of this approach in the actual operation of the assay laboratory. The standard set by this volume, and the list of topics mentioned for review in forthcoming volumes, should ensure a wide and continuing interest in the series.

N. Blakebrough

THE PERIODIC LAW

Periodic Law

By D. I. Mendeleieff. Edited by B. M. Kedrov. Pp. 830 (Russian text). (Moscow: Acad. Sci. U.S.S.R., 1958.) 33 r.

THIS valuable and substantial volume containing Mendeleieff's publications relative to the Periodic Law represents a publication quite independent from Mendeleieff's "Scientific Archives" reviewed in these columns in 1955 (*Nature*, **176**, 965; 1955), the first volume of which was published in 1953, while the second and the third volumes remain as yet unpublished. The present volume belongs to the series "Classics of Science" and is apparently entirely due to the initiative and labour of B. M. Kedrov, assisted by the late T. N. Chentzova.

The first section of the book contains reprints of fifteen "Principal Articles" by Mendeleieff relating to the Periodic Law. Actually the first item of this collection is not an article at all but a copy of the first issue of the Periodic Table (March 1, 1869). Besides this copy the book contains a large number of photographic and printed reproductions of different variants of the Periodic Table.

The second item in this collection is the first article of Mendeleieff dealing with the Periodic Law published in the *Journal of the Russian Chemical Society* (**1**, pp. 60–77; 1869). This is followed by a number of shorter articles and then by the Russian text of the German article entitled "Die periodische Gesetzmässigkeit der chemischen Elemente" published in *Liebigs Annalen*, 1871, Supp. VIII, Heft 2, pp. 133–229. Extracts from this article were published by W. Crooks in the quarterly *Journal of Science*, No. 55, (1877), and a full translation in the *Chemical News*, **40** (1879), and **41** (1880). According to Mendeleieff himself, this article presented "the best summary of my views and arguments on the periodicity of the elements and was the main text used for the numerous comments on this system. This article was the main cause of my renown as a scientist". Short papers on peroxides follow with others on the atomic weight of yttrium, the cerium group of metals, gallium and the rare metals. Number thirteen is the Russian text of the Faraday Lecture entitled "The Periodic Law of the Chemical Elements". The lecture was delivered by Mendeleieff on May 23/June 4, 1889, before the Chemical Society in London (*J. Chem. Soc.*, **55**, 634; 1889). The remaining items are an article from the Russian Encyclopædia of 1898, and an extract from the "Principles of Chemistry", 1906 edition.

The section entitled "Additions" contains a miscellaneous collection of Mendeleieff's articles of lesser

importance, notes, letters and extracts from books, all of them related to the Periodic Law. The items in this section are arranged in groups labelled *A* to *J*. Briefly their contents are: (*A*) collection of periodic tables and other tables of various dates (1869–1906) as they appeared in the successive editions of the "Principles of Chemistry"; (*B*) extract from the first edition of the "Principles" (1870–71); (*C*) a group of three articles, one from *Le Moniteur Scientifique*, **9**, 691 (1879); and two from *Berichte d. chem. Ges.*, **4**, 348 (1879) and **13**, 1796 (1880), dealing with the history of the Periodic Law and the controversy with Lothar Meyer; (*D*), (*E*) and (*F*) are miscellaneous extracts; (*G*) extracts relating to the discovery of the inert gases and radioactivity (1895–1905); (*A*) a collection of various items including the Russian text of the lecture read on May 19 and 31, 1889, at the Royal Institution in London, and entitled "An Attempt to apply to Chemistry one of the Principles of Newton's Natural Philosophy" (*Chemical News*, 40 (1889); (*I*) miscellaneous extracts; (*J*) extracts from Mendeleieff's earlier works (1855–61) relating to the preparatory work in connexion with the future discovery of the Periodic Law.

The last section of the book is entitled "Supplements" (pp. 675–825). It contains a varied assemblage of very valuable editorial notes, commentaries, bibliographical and biographical data, two coloured reproductions of Mendeleieff's portrait and a very interesting article by B. M. Kedrov, "The Periodic Law of Mendeleieff and the World's Science".

S. I. Tomkeieff

X-RAY POWDER METHODS

The Powder Method in X-ray Crystallography

By Prof. Leonid V. Azároff and Prof. Martin J. Buerger. Pp. xv+342. (London: McGraw-Hill Publishing Company, Ltd., 1958.) 68*s*.

THE appearance of this authoritative work on the X-ray powder method is very welcome. It is more compact than some other works that have been produced recently, but is nevertheless exhaustive enough for most research workers, and the extensive references give ample guidance for further reading.

The exposition of basic ideas bears the clear mark of experienced and thorough teaching; the stress laid upon the reflexion concept in Bragg's treatment of X-ray diffraction is particularly noteworthy since so many text-books gloss over this important point. The book also contains ample experimental detail and advice on various techniques to help both the beginner and the more experienced student. The section on interpretation and indexing is particularly good.

The only adverse criticism that might be made is that the book tends to lay down American practice as final and complete. There are several points in which practice differs from that in Great Britain, and the American practice is not always as logical as it is made to appear in this book. For example, cameras in Britain are 9 and 19 cm. in diameter; in the United States they are 5·7 and 11·4 cm. Although there is some arbitrariness in the former figures, they were designed to give films just less than 30 cm. in length since comparators of 30 cm. were readily available. The American radii give a simple number of millimetres per degree; but the reviewer has never met anyone who makes use of this property: for rough work one uses *d* scales directly, and for accurate work film shrinkage must be allowed for.

Secondly, the Americans use the Straumanis design because the camera does not need to be calibrated. But this design is rather awkward, and the calibration of cameras is not particularly difficult. Certainly the Straumanis design does not lead to higher accuracy; the authors claim 2–4 parts in 10^5 as a limit, but Bradley and Jay were achieving this accuracy in 1932.

Thirdly, the Americans make considerable use of accurately fitting slides, whereas British cameras use kinematic supports. Consequently, the former need individual supports for each camera, whereas kinematic design allows for much more flexibility.

These points, however, merely indicate that the subject is a living one, in which there are still many points of divergence between different practitioners. The book can be thoroughly recommended.

H. Lipson

GLOVE BOXES AND SHIELDED CELLS

Glove Boxes and Shielded Cells for Handling Radioactive Materials

A Record of the Proceedings of the Symposium on Glove Box Design and Operation held in the Cockcroft Hall, A.E.R.E., Harwell, February 19–21, 1957. Edited by G. N. Walton. (Sponsored by The United Kingdom Atomic Energy Authority, Harwell.) Pp. xii+515. (London: Butterworths Scientific Publications; New York: Academic Press, Inc., 1958.) 95*s*.; 16.80 dollars.

AS one of the authors remarks, one of the most difficult problems facing the worker with radioactive materials is to decide what degree of protection is appropriate to his particular circumstances. The introductory chapters of this book provide useful basic data and discussions of principles, which should be helpful in determining a logical and realistic policy for the use of glove boxes. The fundamental requirements of radiological protection have not changed appreciably since this Conference was held in 1957, but the reader should bear in mind that controls have tended to become more stringent meanwhile, particularly in the higher ranges of activities.

For alpha emitters and the more toxic isotopes generally, complete containment of the material in a glove box or similar enclosure is essential. The majority of the papers in this symposium are concerned with this aspect, and together they form an extensive review of practice which is now fairly well established. Many aspects of design are covered by contributors with first-hand experience of them, such as materials for construction, radioactive decontamination, filtering systems and the use of inert atmospheres. The papers are mainly drawn from experience within the U.K. Atomic Energy Authority, but there are also some excellent reviews of practice in the United States and other countries. The techniques described here are for use with radioactive materials, but they may also be of interest in connexion with other work where critical control of environment is necessary, as in dust-free or aseptic procedures.

For isotopes which emit penetrating radiations the operations become much more complex, as shielding must be superimposed on the requirement of containment. The choice of system, whether it should be tongs in shielded boxes or manipulators in

cells, is correspondingly more difficult. The second part of the book is concerned with such problems, and it contains a lot of useful data for design as well as practical experience in dealing with highly active materials up to the 1,000 MeV. curie level. It refers particularly to work with fission products and irradiated metallurgical specimens. This part is necessarily rather less satisfactory than the first, because these techniques are at present developing more rapidly than those for handling alpha emitters.

The book is well produced, and one appreciates the heavy task of the editors and publishers in attaining this result. Nevertheless, there is much to be said in favour of adopting a simpler, cheaper and quicker form of publication for the proceedings of symposia such as this one.

MATRIX ALGEBRA IN STRUCTURAL ANALYSIS

Linear Structural Analysis

An Introduction to the Influence Coefficient Method applied to Statically Indeterminate Structures. By Prof. P. B. Morice. Pp. xii+170. (London : Thames and Hudson, Ltd., 1959.) 35*s*. net.

ONE of the most important advances in the theory of elastic structures during the past few years has been the development of matrix methods of analysis. This work has been considerably stimulated by the advent of automatic digital computers, since these machines are very well suited to the manipulation of large matrices.

Methods of structural analysis may be divided into two groups. The first, often termed 'equilibrium' or 'stiffness' methods, consider joint displacements as the basic unknowns, and proceed by setting up and solving the equations of joint equilibrium. The second group, known as 'compatibility' or 'flexibility' methods, attack the problem in terms of the redundant forces and moments in a structure, determining these by equations of displacement compatibility.

Prof. P. B. Morice's book is entirely devoted to the second of these two approaches. He commences by discussing general energy theorems, influence coefficients and indeterminacy. The treatment of the latter subject follows recent work by Henderson and is extremely thorough. The central section of the book presents a short account of matrix algebra and the associated numerical techniques, described from the point of view of a human computer using a desk calculating machine. After this the flexibility coefficient method of analysis is presented in matrix notation, with examples of its use in particular cases. The book concludes with a brief account of the way in which matrix work can be carried out on a *Pegasus* computer.

The development of matrix methods of structural analysis is by no means complete, and the theoretician will doubtless find that many of the ideas presented in this book are capable of further generalization and extension. Although the conciseness and generality of matrix notation are well displayed, there are several places where unusual symbols (such as x and n for force vectors) make the text more difficult than it might have been for a newcomer to the subject. There are also a few misprints which suggest rather hurried proof reading.

From the short title on the cover, and various paragraphs in the preface and introduction, the reader with a limited knowledge of structural analysis might well assume that the best, if not the only, way of using matrices in structural analysis was by means of the flexibility methods described in this book. The book would have been a very much more valuable one if it had contained a parallel account of the equally important stiffness methods of analysis, with demonstrations of the relative merits of the two approaches in a number of examples.

R. K. Livesley

THE GEOLOGICAL DEVELOPMENT OF NORTH AMERICA

The Evolution of North America

By Dr. Philip B. King. Pp. xvii+190. (Princeton, N.J. : Princeton University Press ; London : Oxford University Press, 1959.) 60*s*. net.

THE publication of a book which collates and interprets the considerable number of available facts concerning the regional geology of North America can be expected to be a notable event when the author has the background of Dr. P. B. King, with wide and long experience in the United States Geological Survey and interests covering tectonics, stratigraphy and physical geology. The story of the growth of the northern part of the American continent is not treated chronologically but regionally, with emphasis on selected areas of the United States. Although the book arises from lectures to graduates, the author explains that it is not a text-book in the usual sense ; prominence is given, however, to 'Reference Material', a list of maps and books, which receives precedence over 'Contents' and is supplemented by 'References' (which are not referred to in the body of the text) at the end of each chapter.

After an introductory chapter addressed mainly to the layman, the Canadian Shield is treated in broad outline. When he turns to consider the Interior Lowlands, Dr. King is able to draw on his personal experience of field work in the West Texas Basin, and in the chapter on the Appalachians his close acquaintance with the complexities is evident. Valuable comments on geosynclines and the growth of the Appalachians, stemming from earlier publications by the author, are followed by examination of modern analogues of geosynclines around the Gulf of Mexico and the Caribbean. The second half of the book examines the mountain chains and intermontane regions in the west, largely in the United States. The complex subdivisions of this belt are clearly outlined and interesting notes and anecdotes concerning its exploration are included.

The geological maps and sections are admirably executed, and the sketches by Trudy King above chapter headings add a pleasing touch. The typography can only be criticized in details ; sub-headings tend to become obscured in the text and the list of contents (pp. xi–xiv) suffers from lack of variation in fount and insufficient indentation of sub-headings.

Dr. King succeeds, with a fresh and stimulating approach, in outlining a great deal of the structural framework of North America but is only partially successful in fulfilling the promise implied by his title with respect to historical geology.

J. W. Cowie

Advances in Electronics and Electron Physics

Vol. 10. Edited by L. Marton, assisted by Claire Marton. Pp. x+320. (New York: Academic Press, Inc.; London: Academic Books, Ltd., 1958.) 10 dollars.

A DIVERSITY of subjects has always been a feature of previous volumes of the series of reports on "Advances in Electronics and Electron Physics" (the last three words having been added after Vol. 5). Papers on physical effects and on the preparation and properties of materials have been freely intermingled with others describing the methods of designing, making and using devices and with theoretical analyses. The tenth volume is no exception; indeed, the diversity may be thought to be excessive. Thus, the paper on the logical organization of computers, well written though without recourse to a single diagram or mathematical expression, is perhaps too remote from another which analyses electron flow in magnetically focused cylindrical beams and which is unlikely to attract the attention of anyone not engaged in designing or using microwave valves. Three papers should be widely approved. The first deals with defects in crystals of semiconductors with the diamond lattice, a subject which has benefited from the availability of large crystals of germanium and silicon. The second shows how micro-wave engineers have adapted, and can still further adapt, techniques from optics. The third describes recent progress with the design of cathode ray oscilloscopes (Is this the one remaining use of the words 'cathode ray'?). Partly because their early role of presenting recurrent wave forms has long been extended to transient phenomena, and they are easy to use, these flexible instruments are now found in almost all branches of science, and a few of the arts, and their design, capabilities and limitations deserve the widest recognition. The sixth paper, on the reliability of valves, can for the most part be appreciated only by valve engineers, but the sections dealing with reasons for failure and action to improve life are of more general interest. J. R. TILLMAN

Second Symposium on Coal Preparation, Department of Mining, University of Leeds

21st to 25th October, 1957. Pp. xii+513. (Leeds: The University, 1959. Distributed by the Coal Preparation Plant Association, 301 Glossop Road, Sheffield 10.) 20*s*.

COAL preparation may be regarded as a collective term for those processes, mainly physical and mechanical, to which coal may be subjected in order to make it suitable for specific use. These processes include hand cleaning, hand selection, blending, screening, breaking, crushing, pulverizing, wet cleaning, dry cleaning, de-dusting, dust proofing, drying, de-watering, froth flotation and briquetting. Aspects of several of these processes were discussed at a Symposium held at the University of Leeds, the proceedings of which contain eighteen papers, a detailed record of the discussions on each paper and summaries of rapporteurs on each of the four groups of subjects in which the papers may be classified.

The subjects dealt with range from trends in the pattern of coal preparation to traffic control and wagon marshalling at colliery and coal preparation sidings, and include size distribution equations, sampling, problems of moisture and moisture retention in coals, aspects of certain processes—including dense-medium cleaning and stratification in jig washers, principles and practice of froth flotation, washery water, testing and performance of coal preparation plants, and briquetting.

There is a balanced combination between the various papers of basic principles, research and industrial practice. The publication is a valuable addition to the literature of coal preparation.

S. G. WARD

A History of Western Technology

By Friedrich Klemm. Translated from the German by Dorothea Waley Singer. Pp. 401+24 plates. (London: George Allen and Unwin, Ltd., 1959.) 32*s*. net.

THIS is a book of much interest, but its title is misleading. It is in fact an anthology consisting of 219 passages on technological topics selected from contemporary writings. Since inventors and engineers have not always written about their work or writers concerned themselves with contemporary technological developments, a history based solely on original writings must appear somewhat defective and unbalanced. The compiler, who is the librarian of the Deutsches Museum, Munich, has therefore attempted to give a continuous account by supplying connecting passages and an introduction to each chapter.

The extracts are chosen with the view of depicting the atmosphere in which technological advance took place rather than describing the technology itself. Owing to the lack of earlier material the story only begins with ancient Greece; and, on the other hand, the treatment of the past hundred years is very selective and brief.

The reader is offered much interesting material which he may not know of or trouble to translate for himself. Thus there is Filarete's description of a visit to an ironworks in 1464, the extracts from the proceedings of the Nuremberg Council in relation to the inconvenient inventiveness of two of the red-metal turners living there in the second half of the sixteenth century, and the observations of Fischer, Schinkel, Beuth, and Harkort, German visitors to Britain in the nineteenth century. The book is well illustrated with 59 line drawings and 24 half-tones.

K. R. GILBERT

Principles of Geochemistry

By Prof. Brian Mason. Second edition. Pp. viii+310. (New York: John Wiley and Sons, Inc.; London: Chapman and Hall, Ltd., 1958.) 68*s*. net.

THE second edition of this excellent introduction to geochemistry follows the same general lines as the first, but although no major changes have been made, changes in detail are numerous. Many of the data in the first edition have been replaced where possible by the more reliable figures that have become available during the past six years, and results of recent work have been incorporated in the text. The number of tables and text-figures has been increased and the bibliographies at the end of each chapter have been revised and brought up to date. A new and welcome feature is the introduction of two appendixes, one giving atomic weights, atomic numbers and ionic radii of the elements, the other giving a geological time-scale.

This is a useful text-book for students, and it is unfortunate that the price has had to be increased to such an extent. S. R. NOCKOLDS

THE BOTANIC GARDENS, SINGAPORE, 1859–1959

By H. M. BURKILL

Director, Botanic Gardens, Singapore

IN this year of significance in the political history of Singapore, the Botanic Gardens at Singapore celebrate the centenary of their founding. Centenaries are in the air and they are in good company: their elder sister at Kew has just observed a bicentenary; Kuala Lumpur, the capital city of the Federation of Malaya, in which and for which territory these Gardens have done so much of their work, has this year attained its century, and, if one might stretch a biological association, so has the Guinness Brewery.

The present Gardens are the last of three. The first were set up by Sir Stamford Raffles in 1822, when the East India Company settlement was but three years old. These lasted until 1829 when, on a false notion of economy, they were abandoned. They were resuscitated in 1836 by an Agri-horticultural Society which ran them until 1846. In 1859 another Agri-horticultural Society with official blessing took possession of land which is now a part of the present Gardens.

The intention behind all these Gardens was to foster and encourage the agriculture of the fast-expanding Colony. The first Gardens introduced and grew possible economic crops, but chiefly nutmegs, cloves and cacao. The second Gardens managed to recoup its expenses on the sales of nutmegs and cloves. Though the intention of the 1859 Agri-horticultural Society was also to give encouragement to local agriculture, it set about first creating a pleasure garden. Perhaps this was an economic necessity in order to attract membership, for membership carried privileges. After the pleasure garden got under way, the superintendent, Lawrence Niven, who designed and carried out the landscaping, was able to turn his attention to growing fruit, vegetables and flowers for distribution to members.

The Society soon found itself in financial difficulties, and bit by bit Government assistance became a bigger item in its budget. In 1869, at the invitation of the Committee, the Government appointed a representative to the Committee and increased its contribution on condition the Society exhibited living specimens of economic plants. In order to add popular attraction, a zoo was established in the Gardens at this time, which lasted until 1905. Finally, in 1874 the Committee requested the Government to take over the Society's assets and liabilities and to run the Gardens out of public funds. The Government agreed, and from January 1, 1875, to this day the Gardens have been an official department.

This change in management brought with it a change in purpose. The Government sought to broaden the scope of work to include a study of the flora of the Malay Peninsula, and instructed the committee of management to obtain the services of a botanist who was also a practical man. Henry James Murton was engaged and arrived in Singapore in 1875. In the five years he was superintendent, until 1880, when he was dismissed for neglect of duties, he achieved much, but mainly what interested him. He greatly extended the range of plants grown in the Gardens; he corresponded and exchanged with other Gardens; he travelled in the Malay Peninsula and began a herbarium; he drafted in manuscript a Flora of Singapore. Perhaps the most significant of his works was his handling of the first *Hevea* seedlings to be established in Singapore and Malaya in 1877, the progeny of which in very large measure have stocked the plantations of south-east Asia. He also established many other plants of possible economic importance, and in 1879 he began planting in the land to the north of the Gardens, later known as the Economic Garden and now part of the grounds of the University of Malaya.

Nathaniel Cantley followed Murton, arriving in Singapore in 1880; but he soon became sick and his subordinate, Walter Fox, was left in charge, as he was during the interim between Murton's departure and Cantley's arrival. Cantley returned in 1881, and in the next year the Government commissioned him to survey the forests of the Straits Settlements. As a result, a Forest Department was formed with Cantley as its head and with officers in the three settlements of Singapore, Penang and Malacca, and later in the Dindings. Cantley planted an arboretum in the Economic Garden to have on hand local trees. To these he added exotics. In the Gardens he made great changes in layout and arranged the planting according to taxonomic classification. In 1887 his health broke down and he died.

The appointment of Henry Nicholas Ridley in 1888 as director marked the opening of another chapter in the history of the Gardens. He was a man of tremendous energy and capacity for work, and even though he had an admirable foundation to build on, he pushed the Singapore Botanic Gardens to the forefront of international botany. His interests were manifold. A brief biography by J. W. Purseglove was published in *Nature*, **176**, 1092 (December 10, 1955) on the occasion of his hundredth birthday. Ridley travelled far and wide in Malaya and in the region. His collecting was prodigious and he advanced the knowledge of the Malayan flora to such an extent that when he wrote his "Flora of the Malay Peninsula" (published 1922–25) after his retirement, it filled five thick volumes of 2,948 pages. During the earlier part of Ridley's directorship the Gardens continued to administer the forests of the Straits Settlements, but a separate Forest Department was set up in 1901. The Gardens work on economic crops grew, and agriculture in Malaya developed to such an extent that a separate Agricultural Department was established in 1904; but the Gardens continued to publish an agricultural periodical, begun in 1891, until 1912 (*Agricultural Bulletin of the Malay Peninsula*, 1891–1900; *Agricultural Bulletin of the Straits and Federated Malay States*, 1901–12.)

Perhaps the most spectacular work of the Gardens during Ridley's time was that on rubber. When Ridley arrived he found nine mature trees, some fifty which were 2–4 years old, and more than a thousand seedlings. Ridley experimented on means for their exploitation, persisting even in the face of official disapproval. His tapping technique remains fundamentally unchanged to this day. The Gardens planted *Hevea* in forest clearings and on waste ground, so that when the planting rush came at the beginning of this century the Botanic Gardens was the only major source of supply of seed. Up to 1917, when the Gardens ceased selling seed, more than 7 million had been distributed. Though *Hevea* holds the limelight, the Botanic Gardens have been responsible for the introduction and distribution of many other economic crops.

With Ridley's retirement in 1912, Isaac Henry Burkill, who had been adviser on economic botany to the Government of Bengal, came as director. The First World War hampered development and limited staff, but interest in plants of economic value was maintained. The Agricultural Department naturally held the established economic crops within its purview, but the Gardens continued to be a source of supply of many. A list of plant material available for sale dated 1916 had 200 species, but the loss of the Economic Garden in 1924 for the building of a college for higher education meant the end of this work. During his directorship, I. H. Burkill travelled much, particularly after the war years, and amassed a great quantity of data on native uses of plants. He specialized on *Dioscorea*, native medicines cultivated plants and ethnobotany.

At the end of the First World War, T. F. Chipp was appointed assistant director and he extended the work of the Department to the Cryptogams (fungi); but he soon transferred to West Africa. R. E. Holttum was appointed to the vacancy in 1922, a condition of the appointment being that he should study a group of plants different from that covered by the director. Holttum began a study of the ferns, which he has made a life-work, and now in retirement he is preparing a monograph of the ferns for "Flora Malesiana", a work which will take him perhaps ten years to accomplish.

In 1924 the Gardens benefited by the cessation of botanical work at the Perak Museum at Taiping, and M. R. Henderson joined the staff as curator of the Herbarium. The Perak Museum Herbarium followed him to Singapore and was incorporated with the Singapore Herbarium in 1926.

I. H. Burkill retired in 1925 and at once commenced to write "A Dictionary of the Economic Products of the Malay Peninsula", which eventually took him ten years to complete. Unfortunately, only 2,000 copies were printed and it has long been sold out. Such is its value to all tropical botanists and agriculturists that it is hoped to reprint it.

With the surrender of the Economic Garden in 1925, the Botanic Gardens lost its immediate contact with economic agriculture and forestry, and the work became more concerned with ecology, taxonomic botany and horticulture. In the last field, under the

Fig. 1. Main office buildings. Left: the end of a building erected between 1883 and 1892, now used to house a part of the Library, a store and a 'kitchen' (a room for drying plant specimens over a fire). Centre: the herbarium, housing some 400,000 specimens. The ground floor was built in 1903, and the top floor was added in 1930. Right: administrative office and laboratory, built 1920

Fig. 2. Gardens from inside the main gate. Beds of hybrid cannas flank the road. To the right are collections of bougainvilleas, conifers and casuarinas

directorship of Holttum, a programme of hybridizing was begun, particularly with orchids, which has continued to this day with undiminished vigour. This work has given rise to a thriving orchid nursery industry with an increasing export trade and to an extremely active band of amateur orchid fanciers and breeders throughout Malaya.

In the botanical field, Holttum worked not only on ferns but also on bamboos, gingers and, naturally, on orchids. Henderson specialized on the limestone flora of Malaya, Myrtaceae and *Callophyllum*. He wrote "Malayan Wild Flowers" in two volumes, which was published by the Malayan Nature Society. E. J. H. Corner, who joined the staff in 1929, worked on fungi, trees and ecology, and especially on the genus *Ficus*. C. X. Furtado, who came in 1923, studied palms, aroids and other groups of monocotyledons. Specialist work of the Botanic Gardens was thus well diversified and had in addition the benefit of collaborators such as Carr (orchids), Foxworthy (F.M.S. Forest Department, forest trees), and many others. This period of increased taxonomic study continued up to the outbreak of the Second World War and is recorded by a not insubstantial run of issues of the Botanic Gardens periodical, *The Gardens' Bulletin*.

With the restriction of botanical work during 1942–45, the Botanic Gardens were indeed extremely fortunate to have been sent from Japan successively Profs. Hidezo Tanakadate, of Tôhoku Imperial University, and Kwan Koriba, of the Imperial University of Kyoto, as director. No loss was suffered in the Library or in the Herbarium, thanks to their efforts. Holttum, Corner and Furtado were retained to work in the Gardens, and it was perhaps some compensation that Holttum and Corner, relieved of administrative and executive responsibilities, were able to devote their almost undivided attention to revision and preparation of monographs. There is no doubt that this break afforded Holttum the opportunity which otherwise would not have come to him, perhaps until after retirement, to prepare draft manuscripts for Vols. 1 (Orchids) and 2 (Ferns) of the "Revised Flora of Malaya", which were published after the War; "It is an ill wind . . .". It is significant that, like Holttum, both Ridley and I. H. Burkill completed their major literary works after the close of their official careers.

The post-war years have been marked by shortage of staff. This and the state of terrorism in the Federation of Malaya have severely limited botanical exploration. But now more extensive field-work is possible. Greater use is being made of subordinate staff on plant-collecting assignments. Trained Malayan personnel for appointment in both the botanical and horticultural sections of the Gardens is now available, and there is prospect for regaining a full establishment. This is essential so that the Department may add its vital contribution to the economic development of Malaya and the region. The field is immense. Current estimates for the compilation of "Flora Malesiana", in which project the Botanic Gardens is a major collaborator, run into decades. The achievements of the Gardens' first century have been impressive, but the field for the second century paradoxically is increasing. The more we learn, the more we find we have yet to learn.

INTERNATIONAL OCEANOGRAPHIC CONGRESS

IN spite of the increasing application of scientific discoveries to development of weapons and to enhance national prestige, we can still maintain that science is one of the most international of intellectual activities, and hold that anything we can do to promote co-operation is likely to contribute to better understanding between nations. If not generally true, it must still be so for the study of the oceans, with its very wide and numerous bearings on human welfare, and it seems very appropriate that the first International Oceanographic Congress, which took place during August 31–September 11, should have been held in the United Nations headquarters in New York; also that it should be sponsored by the American Association for the Advancement of Science, which has ably demonstrated the part science can play in raising the level of economic prosperity, and by Unesco, which strives to spread the educational, cultural and practical benefits to all nations. Oceanographers have a tradition of working together, and have a number of intergovernmental councils and scientific associations to help them, but this was probably the first time such a grand attempt had been made to assemble enthusiasts from every aspect of the subject and all parts of the world.

The aim was to get to know and help each other, and the programme was arranged around five main topics: "History of the Oceans", "Populations of the Sea", "The Deep Sea", "Boundaries of the Sea", and "Cycles of Organic and Inorganic Substances in the Sea". Each theme had two morning sessions, with lecturers invited to review contemporary advances rather than the older published work, and in the afternoons the audience divided among four discussions of papers related to the morning topics. The main purpose of the conference seemed to be very well achieved: a great deal of unpublished work was discussed and new ideas freely exchanged. Everyone writing about it will be sure to say how wonderful was the exchange of information between people who knew each other or had read each other's papers. The success was largely due to the efforts of the organizing committee, which persuaded oceanographic laboratories all over the world to send the younger members of their staffs as well as directors, and to the generous financial help of some twenty-two government and private organizations in the United States, which overcame much of the difficulty of travel expenses.

The "History of the Oceans" section dealt with the shape and structure of the ocean basins, the forces involved, the history of sea water, and the origin of life. We begin to know enough about the topography of the Atlantic and Pacific Oceans to form a reasonable overall picture and to know where to study the processes at work. Earthquake activity, vulcanicity, gravity and magnetic anomalies are beginning to be related to topography. The abyssal plains are as interesting as the ridges. We still do not know whether continental slopes are rubbish tips or weathered rock. We were warned against too ready acceptance of generalizations attributing all acid rocks found in dredges to transport by ice. There should be more active studies to see if they could be locally derived, and to see if they can be dated; we were told to be careful about giving them to museums. Another caution, from the biologists, was that while those dealing with living material are very careful about drawing conclusions beyond simple description or regional relationships, palæographers showed less hesitation in making positive deductions about ancient seas. The physicists agreed that the difficulty of finding hypotheses to explain the present circulation of the oceans made it quite fantastic to put forward conclusions about those of earlier climatic periods. There are, nevertheless, very exciting attempts to obtain evidence from the stratigraphy, chemistry, physics, biology and nuclear relationships of the sediments. The same types of evidence, and increasingly penetrating studies of the chemistry of sea water and of its exchanges with the sediments, are also beginning to provide a better framework for judging ideas on the origin of life in the sea.

In "Populations of the Oceans", most of the papers and discussions were on biogeography, distribution and behaviour in relation to physiology and environment, evolution and adaptation, and palæobiogeography. One of the morning lectures was a fascinating unpublished study of the distribution of bottom-living organisms in relation to the time they spent in the pelagic larval stage. It was shown that this time varied with temperature, and that metamorphosis could be postponed by unfavourable conditions. There were a relatively large number of papers from the Soviet Union, dealing mainly with the fish, plankton and bottom-living organisms of the Pacific and Antarctic Oceans. Once or twice there was a hint that some of the more special approaches were defending their territories: ethology tried to disclaim a paper which showed how a squid kept itself afloat.

"The Deep Sea" discussions included the physics and chemistry of circulation, stirring and mixing, the nature and origin of bathypelagic life, the special characteristics of abyssal organisms and the distribution of pelagic-sediment types and nuclear processes in pelagic sediments. During discussion of a Soviet study of turbulent diffusion which confirmed some previous work by the late L. F. Richardson, it was very pleasant to see the charm with which Dr. Henry Stommel, probably the only one there who had talked with Richardson, greeted what might be the only mention of his name in the United Nations headquarters with timely reference to his mathematical treatment of foreign politics, arms and insecurity.

There was plenty of evidence that new ideas on turbulent processes are being profitably applied to the study of vertical and horizontal movements and to better understanding of internal waves. One of the most striking diagrams showed the unexpected complexity of the north and south movements below the Gulf Stream. Even more remarkable was one which contoured velocity in a north-to-south vertical section across the sub-surface 'Cromwell' current below the equator in the Pacific Ocean. It showed a thin ribbon of water some 100 metres thick and 100 miles wide flowing eastwards at about 2½ knots,

mainly between depths of 50 and 200 metres, symmetrical about the equator and known to occur over most of the width of the Pacific Ocean, while all the adjoining water, above, below, north and south, flows in the opposite direction. Interpretation of previous climates from the study of the stratigraphy of the deep-sea sediments seems to be one of the most popular oceanic studies, and there was a strong plea for samples of older sediments from depths well below the level attainable by coring. Quite shallow bore-holes would give information as valuable as the much discussed deep bore. It might be easier to plot previous positions of the equator from the carbonate content of the sediments than to plot previous positions of the poles from evidence of previous magnetism. Most of the geologists seem to feel that there ought to be a much greater depth of sediments in the ocean.

Discussions on "Boundaries of the Sea" dealt with the effects of land masses and types of coast and sea bottom on the plant and animal populations. The rather special conditions in bays and estuaries were emphasized. The physical papers dealt with the near-shore circulation. One of the most striking papers gave a preliminary survey of the very complex thermal structure in the Straits of Gibraltar obtained with the new towed chain of thermistors. Prof. W. H. Munk's morning lecture on the spectrum of sea-level, from ordinary waves to changes in mean sea-level, was one of the most appreciated by the Congress, and the relevant afternoon discussion dealt with a wide range of contributions about effects of pressure, wind, climate and seismic disturbances. Further afternoons were devoted to the interchange of energy between sea and air, and to proposed methods for predicting surface conditions and changes in currents. There was a very interesting account of observations relating surface slicks to internal waves in a shallow thermocline. The internal waves travelled in the same direction as the surface waves and the slicks also moved slowly in the same direction.

The section on "Cycles of Organic and Inorganic Substances" included discussions of the chemistry of sea water, the occurrence of biologically active substances, including vitamins and trace elements, primary production, nutrient relationships, balance between living and dead organic matter, exchanges between the sea and sediments and between the sea and atmosphere. C. E. Lucas and J. H. Steele of Aberdeen and L. H. N. Cooper of Plymouth gave morning lectures. There was enough in this section alone to make a conference.

One of the most striking features of the Congress was its evidence of the expanding interest of geologists in the ocean. Biology, for the first time at such a meeting, probably took second place, and except for the new Soviet expeditions it tended to be concentrated in waters not far from shore. The physics of the sea had a good deal to show for the first time, and, not least significant, it could at last be seen to be making observations to check theories.

The morning lectures will be published, and everyone attending the Conference had preprints of abstracts of all the papers contributed to the discussions, a very formidable volume of 1,027 pages. The simultaneous translations provided by the United Nations staff in English, French and Russian were magnificent. We were able to carry on active discussions with Soviet scientists and occasionally listen to them arguing with each other. The facilities of the United Nations building for a large assembly with discussion groups are quite extraordinary. Most of the 1,200 who registered for the Congress seemed to be staying at the Commodore Hotel only a few blocks away, and there was always someone interesting to talk to. The Soviet research ship *Michael Lomonosov*, and five American ships, *Atlantis*, *Chain*, *Crawford*, *Maury* and *Gibbs* were lying at one pier and the French *Calypso* at another. There was a good programme of social events including a particularly agreeable visit to the New York Zoological Park.

One might penetrate deeper into the science at a smaller meeting, but there could be no better way of getting an impressive though perhaps general idea of the increasing interest and advance in all aspects of the subject. For everyone except the organizers it was in fact rather a holiday, and at the same time a most remarkable opportunity for forming new ideas, correcting old ones and thinking what to do next. There can be no doubt that some of the work begun there will be continued in growing friendliness by scientists all over the world. All marine scientists, and especially those privileged to attend, owe a great debt to Dr. Mary Sears and her organizing committee, to all others who helped, and not least to the United Nations.

G. E. R. Deacon

DEVELOPMENTS IN THERMODYNAMICS

A SYMPOSIUM on Thermodynamics was held during August 20–25 at Fritzens-Wattens, near Innsbruck, Austria, under the joint sponsorship of the Deutsche Bunsen Gesellschaft für Physikalische Chemie, and the International Union of Pure and Applied Chemistry, the latter through its Commission on Thermodynamics, the Sub-Commission on Experimental Thermochemistry and the Sub-Commission on Experimental Thermodynamics. The symposium was under the general direction of Prof. K. Schäfer (president of the Deutsche Bunsen Gesellschaft für Physikalische Chemie, and secretary of the Commission on Thermodynamics) with the local arrangements being handled by Prof. E. Cremer, of the University of Innsbruck. Fifty papers were read, and there were 135 participants, representing seventeen countries, including thirty from North America and twenty from Great Britain.

Experimental Thermochemistry

The first part of the symposium, under the general heading "Experimental Thermochemistry", contained twenty-three papers. Eight of these were studies in bomb-combustion calorimetry. The most novel paper of this group, presented by W. N. Hubbard (Argonne National Laboratory) gave a preliminary account of the successful development of a bomb-calorimeter for combustions in an atmosphere of fluorine gas. The bomb is made of nickel, which is rendered inert to

attack during the combustion process by a careful pre-fluorination treatment, so that the inner surfaces become coated with a stable nickel fluoride film. Combustion experiments of an exploratory character have been made on twenty-five metals and fifteen inorganic compounds, and three-quarters of these immediately appeared to be suitable for accurate calorimetric study.

Two papers described recent studies using the rotating-bomb calorimeter: in this, the bomb is charged before combustion with a suitable solvent, and rotated after combustion to produce a uniform solution of the combustion products in a definite thermodynamic state. The method has been developed over recent years, and has enabled highly accurate combustion data to be obtained for a variety of organic sulphur-containing compounds. D. W. Scott (Bureau of Mines, Bartlesville) described the further application of the technique to organic fluorides and to some organometallic compounds: results obtained on fifteen organic fluorides were presented, and values reported for the heats of formation of lead tetramethyl, lead tetraethyl, and dimanganese decacarbonyl. The application of the method to organic bromides was described by L. Bjellerup (Lund), and the derived heats of formation critically compared with those independently available from heats of bromination. Comparison of the heats of formation from combustion with those from reaction heats measured by Lacher and his associates at Colorado is satisfactory.

The advantages of polythene ampoules over glass ampoules as containers for liquids in combustion calorimeters were discussed by H. Mackle (Belfast); in the discussion, it transpired that several investigators have experimented with plastic ampoules with considerable success, and that polythene and polyesters are suitable materials to use. Three papers reported new combustion studies by conventional 'static-bomb' procedures: J. D. Cox (National Chemical Laboratory, Teddington) presented results on phenol and the three cresols; M. Colomina (Madrid) gave the results of an investigation of five alkylphenylketones and of the three toluic acids; and J. Tjebbes (Lund) reported on the heats of combustion of butanal and five related compounds. In these studies, the error limits were of the order 0·01–0·02 per cent, which is fairly representative of the degree of precision attainable by modern combustion calorimetric techniques in favourable cases. C. T. Mortimer (Keele) reported heat of combustion data on triphenylphosphine, triphenylphosphine oxide, and triethyl phosphate. These compounds can scarcely be described as 'favourable cases' for combustion study, in view of the difficulty of analysing the products of combustion, so that the error limits of only 0·1 per cent reported by Mortimer are encouragingly small, and suggest that further thermochemical studies on organophosphorus compounds of biochemical importance should be rewarding.

Reaction calorimetry (heats of reactions other than combustion) was represented by three papers: E. J. Prosen (National Bureau of Standards, Washington) gave an account of the extensive studies on the thermochemistry of boron hydrides, including the measurement of the heats of thermal decomposition of the boron hydrides into elements, of their heats of hydrolysis, and heats of chlorination. The heat of formation of boron trichloride from elements has also been measured. Reliable values are now available for the heats of formation of the boron hydrides and of boric acid. Values were derived for the bond-energy terms of various boron bonds, including the tricentric 'bridge bonds' involving boron atoms. The empirical scheme proposed by Prosen was applied to interpret structural features of the more complex hydrides of boron. H. A. Skinner (Manchester) described some thermochemical studies on reactions of diborane, in this case the addition reactions of diborane with olefines to form boron tri-alkyls. These reactions occur rapidly in diglyme solvent at room temperature, and provide a promising route to the heats of formation of the boron tri-alkyls. N. N. Greenwood (Nottingham) described the application of solution calorimetry to the measurement of heats of formation of 1 : 1 and 1 : 2 donor–acceptor complexes of gallium trichloride, gallium tribromide, boron trichloride and boron tribromide with various oxygen and nitrogen donors. For a given ligand, the heats of formation of the tribromide complexes are consistently greater than for the corresponding trichlorides, and the heats of reaction of boron trichloride with nitrogen donors are larger than those of gallium trichloride with the same ligands.

Two papers dealt with the thermochemistry of alloys. O. Kubaschewski (National Physical Laboratory, Teddington) reviewed calorimeters suitable for measuring the heat of formation of alloys, with particular reference to calorimeters developed at the National Physical Laboratory for direct measurements on solid alloys formed by heating powdered metal mixtures at high temperatures. A. Schneider (Göttingen) reported directly measured heats of formation of InSb, $MgZn_2$, MgZn and Mg_2Zn_{11}, and indirectly determined free energies of formation of Fe_2Zr, Al_2Zr, Al_3Zr_2 and Al_3Zr_4.

There were three contributions concerned with the heats of mixing of liquids. In one of these, M. L. McGlashan (Reading) emphasized that measurements on heats of mixing should be carried out under conditions in which the vapour phase is entirely eliminated, and described an ingenious calorimeter for this purpose. Heats of mixing of pairs of compounds from the series $Si(CH_3)_{4-n}Cl_n$, and from the series $C(CH_3)_{4-n}Cl_n$ were reported, and discussed in terms of the 'quasi-chemical approximation'. Mixing heats in the binary and ternary systems formed from acetic acid, pyridine and a normal paraffin hydrocarbon (hexane, heptane, etc.) were reported by W. Woycicki (Warsaw). W. Wagner (Dresden) described a series of measurements of activity coefficients, heats of mixing and volumes of mixing of the chlorotoluenes in benzene solvent.

Studies of chemical equilibria were the subject of two papers: in one of these, S. Sunner (Lund) described the use of the gas-phase chromatographic technique to analyse the disproportion reaction MeSSMe + EtSSEt $\rightleftharpoons$ 2MeSSEt, at 25° C. and 60° C. A. Pacault (Bordeaux) spoke of a series of equilibrium studies on double decomposition reactions in the solid state between barium sulphate and sodium carbonate and potassium carbonate.

The thermochemistry of tellurium carbonate, tellurium thiocarbonate and barium thiocarbonate, determined from dissociation pressures measured over a temperature range, was described by G. Gattow (Göttingen), and the formation and thermodynamic stability of mixed halides, for example, CuClBr, by R. Perret (Dijon).

I. Wadsö (Lund) described a novel adiabatic calorimeter for the direct measurement of heats of

vaporization at 25° C. suitable for liquids having a vapour pressure of 5–100 mm. mercury at room temperature. The accuracy achieved was of the order of 0·5 per cent.

The accepted value for the heat of dissociation of nitrogen was questioned by G. Glockler (Durham, N.C.), and L. H. Long (Exeter) raised the interesting problem of the relation between bond dissociation energies and valence-state excitation energies, by specific reference to experimental data available on metal–carbon bonds.

This first part of the symposium was summed up by F. D. Rossini (Pittsburgh) and by J. Coops (Amsterdam). Prof. Rossini, reflecting on thirty years of thermochemistry, recalled the stages in its development. First, to design calorimeters of sufficient accuracy; then to prepare compounds of sufficient purity, and now to extend the range of investigation to substances more difficult to prepare, to purify, and to analyse, requiring full use of all the tools of modern analysis.

Physical Constants

The second part of the symposium, arranged by the Commission on Thermodynamics, contained six papers. The first of these, given by F. D. Rossini (Carnegie Institute, Pittsburgh), concerned the present state of knowledge on the fundamental constants of physical chemistry. Five of these have to be determined by experiment; the others are defined by international agreement. In addition, there are auxiliary constants which derive from the fundamental constants. Since 1951, the ice-point of water has become a defined constant, and improved values have become available for the constants, h, c, e, F and N. In 1960, it is proposed to adopt a new scale of atomic weights based on carbon-12 = 12·0000, which will differ from the present chemical scale by 42 p.p.m., and from the present physical scale by 317 p.p.m.

There were three papers on the thermodynamic scale of temperature, and its realization through the international scale, given by Van Dijk (Leyden), H. Moser (Braunschweig) and C. Tingwaldt (Berlin).

In the range 0–90° K., new measurements have been made on the boiling-points of helium and oxygen, with results which differ by only 1/1,000° and 1/100°, respectively, from the older values of 1937 and 1927. The points 4·215° K. and 90·18° K. (relative to the ice-point = 273·15° K.) representing the boiling-point of helium and oxygen may be regarded as in practically complete agreement with the thermodynamic scale. For n-H_2, the most recent measurements of the boiling-point by various investigations gives the mean value 20·379° K. Secondary fixed points in this region are shown in Table 1.

In the range between the two fixed points of the boiling-point of oxygen (− 182·97° C.) and the boiling-point of sulphur (440·60° C.), which is spanned in the international scale by measurement

Table 1. Secondary Fixed Points

Gas	Boiling-point (° K.)	Triple-point (° K.)	λ-point (° K.)
Argon	83·81		
Nitrogen	77·35		
Oxygen		54·34	
p-Hydrogen	20·27	13·81	
n-Hydrogen		13·95	
Helium			2·172

using the platinum resistance thermometer and the three-term formula, the deviations $\Delta T(=T$ (thermodyn.) $-T$ (internat.)) vary from +0·04° at −80° C. to + 0·13° at 400° C. In the range from the boiling-point of sulphur to the melting-point of gold, in which the international scale relies on the interpolation formula for the e.m.f. of a platinum/platinum–rhodium element, the deviations ΔT reach as high as 1·7° C. It is suggested on the basis of new measurements that the melting-point of gold is 1,064·76° C. in place of 1,063°, as accepted by the international scale. This is a large change to make, and until more evidence is available it is premature to make the change in the international scale.

A change in the radiation constant from 1·438 to 1·4388, coupled with an increase of 1·76° C. in the defined melting-point of gold, would eliminate the differences between the international and thermodynamic scales above 1,500° K.

The measurement of temperatures above the gold point by optical pyrometry was discussed by C. Tingwaldt, with particular reference to experimental methods of obtaining ideal black-body radiation from a glowing solid. The lateral radiation from a glowing metal is extensively polarized 'non-black' radiation: by allowing radiation from one source to fall at an angle of 45° on to a glowing metal strip, the combined radiation (reflected and emitted) from the strip is unpolarized, black radiation corresponding to the temperature of the glowing source.

There were two papers describing new designs of calorimeter. One of these, by A. R. Meetham (National Physical Laboratory, Teddington) reported on the design and use of an aneroid bomb-calorimeter made of silver, and vacuum-jacketed, for measuring heats of combustion. The heat transfer coefficient of the calorimeter is very much smaller than that of stirred-water calorimeters. The internal volume is 97 ml., and 0·2 gm. benzoic acid on combustion gave a 3·5 deg. C. temperature rise. The standard deviation of individual estimates of the heat of combustion of benzoic acid was less than 1 part in 5,000.

F. E. Wittig (Munich) described the constructional details and operation of a high-temperature calorimeter for thermal studies on metallic alloys.

Phase Changes in Condensed Systems

The third part of the meeting had as its subject "The Thermodynamics of Phase Changes in Condensed Systems", and was sponsored by the Sub-Commission of Experimental Thermodynamics. Introducing the first session, Prof. D. M. Newitt (Imperial College of Science and Technology, London) said that the object of this meeting, and of its predecessor in London in 1957, was to encourage work to fill the gaps in our knowledge of thermodynamic properties, and especially of those of pure substances.

The majority of the speakers discussed the phase transitions of solids. There were two introductory reviews. The first was by J. E. Mayer (Chicago), who gave a lucid account of the thermodynamics of phase changes in terms of the singularities of the Helmholtz free-energy and emphasized how restricted were the types of transition that could be adequately treated by the present developments of statistical mechanics. The second was by J. P. McCullough (Bureau of Mines, Bartlesville), who outlined an empirical division into seven types of the solid–solid transitions of hydrocarbons and of related substances.

A. R. Ubbelohde and his colleagues, Miss E. Rhodes and E. R. Buckle (Imperial College of Science and Technology, London), contributed a group of three papers which discussed the mechanism of melting and freezing. They demonstrated most convincingly that the classical thermodynamic picture of melting as the sharp intersection of two unrelated free-energy curves was inadequate for material composed of complex molecules or ions.

The largest group of papers in this section were detailed studies of particular solid systems. Silver oxide was shown by K. S. Pitzer (Berkeley) to have two anomalous regions in its heat-capacity curve. The first, in the region of 20–40°K., was still without a verified explanation, but the second, 370–470°K., was an annealing of surface and crystal defects. J. G. Aston (Pennsylvania State University) and E. F. Westrum (Michigan) both presented measurements of the heat capacities of systems containing highly symmetrical organic molecules, the *molécules globulaires* of Timmermans. Such systems are of particular interest since the symmetry and 'smoothness' of the molecules allow them to melt with respect to their orientations without disrupting the crystal. This orientation energy and entropy are acquired at one or more transitions below the true melting point. J. E. Spice (Liverpool) reported a detailed study of mixtures of ethylene dichloride and dibromide. The former has long been known to have a broad maximum in its heat-capacity curve at 180° K. On dilution with dibromide the peak moves at first to lower temperatures and becomes less pronounced. The behaviour of the system is, however, complicated by the partial immiscibility of the two solids.

The thermodynamic studies of indane and indene by D. R. Stull (Dow Chemical Co., Michigan) were most notable for the high degree of 'automation' in his calorimeter. This led to many questions regarding the time taken to reach equilibrium in many solid systems.

The magnetic transitions of the divalent salts of manganese, iron, cobalt and nickel were reviewed by J. W. Stout (Chicago). These systems provided unusually elegant examples of the entropy ($R \ln n$) associated with n available electronic states per ion. W. E. Wallace (Pittsburgh) has made a very detailed study by calorimetry and by electrical, magnetic and X-ray examination of the differences between the α and β phases of tantalum hydride and tantalum deuteride. The results were interpreted in terms of differing amounts of short-range and long-range order in the allocation of the hydrogen atoms to the interstitial sites. G. M. Schwab (Munich) reported a study of the α to β phase change in cobalt.

The last group of papers were those that dealt primarily with liquid phases, or in which the interest was in the application of theories of mixtures to the results. R. Heastie (Queen Mary College, London) had studied the phase equilibria of mixtures of krypton with argon and with xenon. The systems were miscible in both solid and liquid phases at the melting-point and the form of the melting curves provided a test for the lattice theories of solution of Prigogine. Unfortunately, true equilibrium in the solid phases was not always achieved. T. M. Storvick and J. M. Smith (North-Western University, Illinois) have studied the thermodynamic properties of mixtures of hydrocarbons and alcohols in liquid and gaseous phases at temperatures up to the gas–liquid critical points. The deviation of their results from those of purely hydrocarbon mixtures was interpreted in terms of the degree and heat of polymerization of the alcohol molecules. J. S. Rowlinson (Manchester) demonstrated that lower critical solution points are not confined to polar mixtures but are commonly found also in binary hydrocarbon mixtures if the size ratio of the constituent molecules is sufficiently large. This behaviour appears to be the rule for high-polymer solutions, almost all of which separate into two phases above the normal boiling-point of the solvent. Finally, R. I. Munn (Vienna) reported a careful re-examination of the phase-boundary curve near the lower critical solution point of the system water + triethylamine. The curve has an unusual, and unexplained, point of inflexion.

Three papers less easy to classify were given by K. S. Pitzer on "Irreversible Thermodynamics", D. White (Columbus, Ohio) on "*o-p* and Isotope Separations by Preferential Adsorption at Low Temperatures", and by G. Watelle-Marion (Dijon) on a spectrophotometric study of the ionization of a divalent–metal salt.

H. A. Skinner
K. Schäfer
J. S. Rowlinson

OBITUARY

Prof. F. Kögl

Prof. F. Kögl died on June 6 at Utrecht after an illness of about two years. From 1930 until his death at the early age of sixty-one, he had been professor of organic chemistry and of biochemistry at the State University of Utrecht.

Fritz Kögl was born on September 19, 1897, at Munich. He studied chemistry under such famous teachers as H. Wieland, H. Fischer and A. Windaus and became well known himself at an early age by his studies of natural pigments produced by certain bacteria and fungi. While a *Privatdozent* at Göttingen, he was called to a professorship at Utrecht in 1930 as the successor of Van Romburgh and of Ruzicka. Here he widened his field of activities enormously. Interest in growth phenomena of plants led to the isolation and characterization of the auxins. He and his co-workers were the first to recognize β-indolylacetic acid as a natural plant-growth hormone. The consequences of this work are well known to all dealing with the theoretical and practical aspects of plant growth. His studies of growth factors for micro-organisms led to the isolation and the partial characterization of biotin.

From normal growth processes Kögl turned his attention around 1939 to the abnormal growth occurring in animal tumours. He developed a daring hypothesis based on a presumed loss of stereo-specificity, resulting in the incorporation of D-amino-acids in tumour proteins. Although this theory could not be maintained against experimental evidence, it proved very stimulating for the continued biochemical approach to protein chemistry. During

the past ten years many new fields of current biochemical interest were tackled. Among these, important studies on diabetes, on membrane chemistry and on thermo-regulation in the warm-blooded animal must be mentioned.

It was a great satisfaction to Kögl himself that a problem started before 1930 was solved just before his final illness. This was the elucidation of the chemical structure of muscarin, the poisonous constituent of the fly-agaric (*Amanita muscaria* L.). The structure of muscarin had puzzled chemists for more than a century; Kögl and his associates concluded this chapter brilliantly by synthesizing the active compound.

Kögl was an inspired investigator and teacher. More than sixty doctoral theses are a lasting testimony of the School he founded. His death is a grievous loss to the University he served for almost thirty years. He is survived by his wife and two daughters.

G. J. M. van der Kerk

NEWS and VIEWS

Royal Society: Officers for 1960

At the anniversary meeting of the Royal Society, Sir Cyril Hinshelwood, Dr. Lee's professor of chemistry in the University of Oxford, was re-elected president. The other officers re-elected for the ensuing year were: Treasurer, Sir William Penney, member for scientific research, United Kingdom Atomic Energy Authority; Biological Secretary, Sir Lindor Brown, Jodrell professor of physiology at University College, London; Physical Secretary, Sir William Hodge, Lowndean professor of astronomy and geometry in the University of Cambridge; and Foreign Secretary, Dr. H. G. Thornton, lately head of the Department of Soil Microbiology at Rothamsted Experimental Station. Other members of Council elected were: Prof. J. F. Baker, professor of mechanical sciences, University of Cambridge; Dr. J. C. Burkill, University lecturer in mathematics, Cambridge; Prof. D. G. Catchesides, professor of microbiology, University of Birmingham; Prof. T. M. Harris, professor of botany, University of Reading; Prof. L. Hawkes, emeritus professor of geology, Bedford College, University of London; Prof. A. L. Hodgkin, Royal Society research professor; Sir Patrick Linstead, rector of the Imperial College of Science and Technology, London; Dr. R. A. Lyttleton, reader in theoretical astronomy, University of Cambridge; Prof. H. S. W. Massey, Quain professor of physics at University College, University of London; Prof. R. A. Morton, Johnston professor of biochemistry, University of Liverpool; Sir Alfred Pugsley, professor of civil engineering, University of Bristol; Prof. R. J. Pumphrey, Derby professor of zoology, University of Liverpool; Prof. H. W. B. Skinner, Lyon Jones professor of physics, University of Liverpool; Prof. W. Smith, professor of bacteriology at University College Hospital Medical School, University of London; Dr. H. W. Thompson, university reader in infra-red spectroscopy, Oxford; and Prof. J. Z. Young, professor of anatomy at University College, University of London.

National Chemical Laboratory: Dr. D. D. Pratt, C.B.E.

After thirty-four years as a member of the staff of the National Chemical Laboratory, Dr. D. D. Pratt retired from the post of director of the Laboratory on September 30, but in order to assist the new director, Dr. J. S. Anderson, he agreed to occupy the vacant post of assistant director for a period of two months. Born at Anstruther, Fifeshire, Dr. Pratt graduated at St. Andrews and received his post-graduate training both there and at Manchester under Prof. (now Sir) Robert Robinson. This period was interrupted by the First World War, throughout which he served as an officer in the Highland Light Infantry. In November 1925, a few weeks after its opening, Dr. Pratt joined the Chemical Research Laboratory, being appointed to take charge of research on tars, particularly those from the low-temperature carbonization of coal; this work had several successful applications in industry. His ability was soon recognized by Dr. G. T. Morgan, who quickly promoted him until he was the senior officer under the director. When Morgan retired in 1938, Pratt was made officer-in-charge of the Laboratory for the six months which elapsed before Dr. G. S. Whitby became director. This was the first of several occasions when he was in charge under various titles, until finally he was appointed director in March 1951. His period in this office has been marked by considerable activity and expansion. The rapid increase in radiochemical work led to the creation of a separate laboratory for this purpose. The Microbiology Group was re-housed in a new building, while responsibility was also assumed for the National Collection of Industrial Bacteria. Under Dr. Pratt's leadership the Laboratory has grown in numbers, in size and in reputation. Its name has been changed to the National Chemical Laboratory, there has been some re-organization of the programme; but in spite of growth and change, Dr. Pratt succeeded in maintaining the friendly atmosphere which has always characterized the Laboratory. A friendly man himself, his numerous friends will join in wishing him many happy years of retirement.

Prof. J. S. Anderson, F.R.S.

Prof. John Stuart Anderson, professor of inorganic and physical chemistry in the University of Melbourne, has been appointed director of the National Chemical Laboratory of the Department of Scientific and Industrial Research at Teddington, in succession to Dr. D. D. Pratt. Prof. Anderson, whose work in Great Britain and in Australia has placed him among the leading workers in the field of inorganic chemistry, is particularly well known for his contributions in the fields of solid-state chemistry and the chemistry of complex salts. He was elected a Fellow of the Royal Society in 1953. Prof. Anderson was born in London in 1908. He graduated with first-class honours in chemistry at the Imperial College of Science and Technology, London, in 1928. After three years postgraduate research under Prof. H. B. Baker, he went to Heidelberg for one year. In 1932 he joined the staff of the Chemistry Department at the Imperial College. Six years later Dr. Anderson was appointed senior lecturer in chemistry in the University of Melbourne.

During the War he took part in chemical work aimed at developing new natural resources in Australia. He returned to Britain in 1947 to join the Chemistry Division at the Atomic Energy Research Establishment, Harwell, but went back again to Australia in 1954. Prof. Anderson is joint author with Prof. H. J. Emeléus of "Modern Aspects of Inorganic Chemistry".

Directorship of Colaba and Alibag Observatories : Mr. S. L. Malurkar

Mr. S. L. Malurkar, who has retired from the directorship of the Colaba and Alibag Observatories, was born in Hassan on August 15, 1903, and educated in government schools and colleges at Bangalore. Due to Prof. M. Venkatrama Iyer's influence, he turned to original studies in mathematics after 1920, in addition to the subjects in physical sciences taken in the Central College at Bangalore. His studies included ellipsoidal wave functions, producing many new results. These led to a study of non-linear integral equations for a large number of types of functions, and their evaluation in terms of elliptic functions, asymptotically and otherwise. At Cambridge (1927–29), while working in the Solar Physics Observatory, he published a classification of arc spectra of antimony and arsenic. His stay at Cambridge was initially helped by the Government of Mysore at the instance of Dr. E. P. Metcalfe and the late Sir Mirza M. Ismail. He joined the India Meteorological Department in 1930, taking over the investigations on radiation; by this means he examined temperature distributions near heated surfaces, in collaboration with Dr. L. A. Ramdas. While at Agra (1936–37) he developed a basic theory of balloons for meteorological purposes. In 1948 he was put in charge of the Colaba and Alibag Observatories, where he carried out much important work on geomagnetism and cognate phenomena, the magnetogram and the photolysis of atmospheric ozone in the presence of water vapour. He was president of the Physics Section of the Indian Science Congress held at Madras in 1958.

Royal Society of South Australia Award : Dr. C. G. Stephens

The Verco Medal is the highest award which the Royal Society of South Australia can bestow on scientific workers who have made exceptional contributions in their particular field of interests. This year the Medal has been awarded to Dr. C. G. Stephens for his excellent and outstanding work on soils. Dr. Stephens attended the Launceston High School, following which he attended the University of Tasmania, where in 1929 he graduated, and later gained his master's degree. He was awarded a D.Sc. by the University of Adelaide in 1950 for his work on Australian soils. He joined the Commonwealth Scientific and Industrial Research Organization—then known as C.S.I.R.—on a postgraduate scholarship in April 1929 and has remained with the Organization since that date. His association with its Tasmanian staff ceased in 1938 when he was transferred to South Australia. To-day, he is head of the Soil Survey and Pedology Section, Soils Division, Adelaide. The practical applications of Dr. Stephens's work have important bearing especially in the relationships of soil to land uses and development, and to soil fertility problems. In consequence, he has been asked to give assistance in these matters both within Australia and overseas. Dr. Stephens was appointed consultant at the Food and Agriculture Organization headquarters in Rome in 1952. During this period he added to his publications with the report, "Soil Survey for Land Development". In 1954 he was appointed president of Commission 5 (Soil Classification) at the International Congress of Soils at Leopoldville, Belgian Congo. Dr. Stephens is prominent among Fellows of the Royal Society of South Australia and served two periods as vice-president, namely, 1954–55 and 1956–57, and was president during the year 1955–56.

Higher Education in East Africa

In reply to a question in the House of Commons on November 24, the Secretary of State for the Colonies said that work had begun on the new technical institute building in Nairobi with the help of a £65,000 grant from Colonial Development and Welfare Funds. The East African Governments had accepted the recommendations of the Working Party on Higher Education in East Africa for a University of East Africa, comprising three inter-territorial university colleges, of which the re-constituted Royal Technical College would be one, and they were now studying the financial and other implications of the recommendations with the view of preparing a phased programme acceptable to all three territories.

Petroleum Industry Centenary

The September issue of *The British Survey*, published by the British Society for International Understanding, is a survey of the petroleum industry from 1859, the year that Edwin Laurentine Drake drilled his historic well at Oil Creek, Titusville, Pennsylvania, U.S.A., until the present time, the centenary year of the petroleum industry, which to-day is the biggest industry in the world. The survey traces the evolution of the industry from its origins, but also stresses the economic aspects as affecting not only peoples of the producing countries but also those who consume rather than produce petroleum and its multitudinous products—now well over a thousand in the domestic field alone, quite apart from the more widely known, major refined products. This article is not merely a re-statement of time-worn facts regarding the oil industry and its international ramifications. The sections on "Oil Industry as a Citizen", "Cost, Need and Complication", and "Tendencies to State Participation" are refreshingly original and logically sound. The survey concludes with a modest forecast of the rate of increase in demand for oil in the next 10–15 years: United States, 4–5 per cent per annum; rest of the Free World, 6–7 per cent per annum; Western Europe, 7 per cent per annum. So far as can be estimated at present, the crude oil production to the end of 1958 amounted to the staggering figure of nearly 19 million barrels a day.

Soviet Scientific Information in the United States

The National Science Foundation, Office of Science Information Services, has issued a revised edition of a pamphlet, "Providing U.S. Scientists with Soviet Scientific Information", which presents a concise picture of what is being done to make scientific periodicals, serials and books published in the U.S.S.R. readily accessible to scientists in the United States (pp. 36. Washington, D.C.: National Science Foundation, 1959). The magnitude of the problem is displayed in four tables which give a total of 2,026

periodicals and serials and some 55,732 books in 1955. A selected list of bibliographies published in the U.S.S.R. and a list of dealers in Soviet publications in the United States follow, and the arrangements made for translating Russian periodicals, papers, monographs and abstracts are then briefly described. This account is supported by a table listing Russian scientific periodicals available in English and giving the agency, sponsor and subscription. Some details of abstracting services are included, and there is a list of annual publications which contain reviews of Soviet science.

Geodetic Marker at the South Pole

During the 1960 season of the U.S. Antarctic Research Programme the Coast and Geodetic Survey of the U.S. Department of Commerce will erect a marker permanently identifying a geodetic position near the South Pole. The 3-in. bronze disk to be erected inside a building will indicate the exact location of the South Pole Station of the U.S. Antarctic Research Programme at lat. 89° 59′ 43·6″ S. and long. 24·8′ W. At this spot the scientific leader, Major Palle Mogensen, made 52 independent sets of astronomical observations during the antarctic night. Data resulting from these observations were brought back to the United States by Major Mogensen and passed on to the Coast and Geodetic Survey for computation of the exact position of his observing station. Scientists of the Bureau have determined that the South Geographical Pole lies approximately 1,650 ft. south of Major Mogensen's station.

Vegetation on Mars

It has often been suggested that the dark areas of Mars consist partly of vegetation, particularly in view of the seasonal variation of the intensity of the dark regions. Tests for the high near-infra-red reflectivity characteristic of many plants have all given negative results. A few terrestrial plants, such as some lichens, do not show this characteristic, and possibly such plants are present on Mars. W. M. Sinton (*Astrophys. J.*, **126**, 231; 1957. *Lowell Obs. Bulletin*, No. 103; 1959) has suggested and twice carried out a new test for the presence of vegetation. All organic molecules possess strong absorption bands at wave-lengths near 3·4μ, the wave-length of the carbon–hydrogen bond resonance; the precise wave-length depends on the individual molecule. These bands have been studied by Sinton as they appear in the spectra of a number of terrestrial plants, including lichens and mosses. In all the plants tested, the carbon–hydrogen resonance bands appeared to some extent. Tests were carried out on Mars at the oppositions of 1956 and 1958. Relatively little light is received from Mars at a wave-length of 3·4μ, so that very sensitive equipment was needed to render the work possible. A lead sulphide cell cooled in liquid nitrogen was chosen as the detector and preceded by a monochromator equipped with a lithium fluoride prism. The 1956 tests were made at Harvard and the 1958 tests at the 200-in. telescope at Mount Palomar. The radiation received from Mars was analysed theoretically into thermal radiation and reflected solar radiation. The latter shows three absorption bands at 3·43μ, 3·56μ and 3·67μ. These appear to be peculiar to the dark regions of Mars. The bands are weak or absent in the bright regions, and are therefore formed neither in the atmosphere nor in the bright regions of Mars. No other new absorptions were found. Although one cannot be certain that no inorganic molecule can explain these absorption bands, the observed spectrum does fit very closely that of organic compounds and plants. The first two bands are easily observed in plants; that at 3·67μ seemed at first to be an enigma. However, it has now been found in the alga *Cladophora* and appears to be produced by carbohydrate molecules in the plant. Sinton's results are the best evidence yet produced for the existence of vegetation on Mars.

A New Fossil Penguin from Australia

A specimen of part of a fossil penguin named *Anthropodytes gill* sp. nov., found by E. D. Gill in 1950 in western Victoria, Australia, has been described by G. G. Simpson (*Proc. Roy. Soc. Victoria*, **71**, 113; 1959). The actual find (now housed in the National Museum of Victoria) consists of the right humerus, nearly complete, but lacking most of the head. *Anthropodytes* does not show any unmistakable signs of special affinity with any previously named genus, but of the fourteen known genera of fossil penguins in which the humerus is known the closest resemblance is to *Archæospheniscus* from New Zealand. Although *Anthropodytes* was not found *in situ*, evidence relating to attached fossils, situation, and the fluorine test presented by E. D. Gill (p. 121, *loc. cit.*) strongly suggest that the specimen came from an adjacent marine Miocene formation.

Molluscan Fauna of the Netherlands

The Netherlands' Malacological Society, Amsterdam, has this year celebrated its twenty-fifth anniversary, and to commemorate the event the Society has published a special supplement to its journal (*Basteria*, Vol. 23) devoted to the Netherlands as an environment for molluscan life. The book contains seven chapters, all in English, with summaries in Dutch. The opening section deals with the history of the study of the Dutch recent Mollusca and this is followed by accounts of the general ecology of the marine, brackish-water, fresh-water and terrestrial mollusc fauna in the Netherlands. Two other chapters are concerned with the nudibranch molluscs and with the dead shells which are found washed up on the beaches. The authors who have contributed to this volume are all well-known authorities on their subjects and the result of their efforts makes good reading not only for those specifically interested in the Mollusca but also for all zoologists who are interested in ecology.

The Agricultural Institute of Ireland

The Council of An Foras Talúntais (The Agricultural Institute), as part of its educational activity in the promotion and encouragement of agricultural research, announces the award of junior postgraduate agricultural research scholarships for the academic year 1959–60 to the following candidates, who will undertake postgraduate studies in the University departments listed: Mr. James Coyle (University College, Dublin), at the Department of Chemistry at the University of Leeds; Mr. Julian Hart (University College, Cork), at the Department of Statistics, University College, Cork; Mr. James Kavanagh (University College, Dublin), at the Department of Botany, University College, Dublin; Mr. Ronan F. Lambe (University College, Galway), at the Department of Chemistry, University College, Galway; Mr. John L'Estrange (University College, Dublin), at the

Department of Agricultural Chemistry, School of Agriculture, University College of North Wales; Mr. Brian T. Little (University College, Dublin), at the Department of Industrial Microbiology, University College, Dublin; Mr. Cornelius Long (University College, Dublin), at the Department of Experimental Physics, University College, Dublin; Mr. Vivian Timon (University College, Dublin), at the School of Agriculture, Newcastle upon Tyne (University of Durham).

Ciba Fellowships

SEVERAL Ciba Fellowships will be awarded for tenure during the academic year 1960–61 at Continental universities or institutions for research in chemistry, physics or some other allied scientific subject. They will be awarded to graduates of universities of the British Isles. The basic award for Fellows who wish to continue postdoctorate studies will be £800 per annum plus allowances. For candidates who have obtained a first degree in science and wish to undergo training in research, the basic award will be £500 per annum plus allowances. The total value of each fellowship will be determined by the advisory panel, and will be sufficient to cover the Fellow's living and other expenses (including fees and cost of travel) during the tenure of his fellowship, which will be for one year, renewable for a further one or two years. Six Fellowships were awarded last year for tenure in 1959–60, and Ciba Fellows are at present working at Zurich, Göttingen, Louvain, Heidelberg and Copenhagen. Applications should be made in duplicate on forms obtainable from the Secretary to the Ciba Fellowship Trust, Ciba (A.R.L.), Ltd., Duxford, Cambridge. The closing date for such applications is January 31, 1960.

University News:

Belfast

THE appointment of Dr. F. J. Wallace (Birmingham) to senior lecturer in mechanical engineering is announced, also L. J. Clein (Maudsley Hospital, London) to be lecturer in mental health.

Bristol

THE following appointments are announced: Dr. J. W. Barton (Bristol), lecturer in organic chemistry; Dr. D. L. Dineley (Birmingham), lecturer in geology; Dr. K. R. Gough (Bristol), research fellow in medicine; Dr. S. Griew (London), lecturer in psychology; Dr. M. A. Hollingsworth (London), lecturer in mechanical engineering; B. S. Smith (Manchester), lecturer in civil engineering; D. J. Storey (Cambridge), lecturer in electrical engineering; Dr. C. J. Waddington (Bristol), lecturer in physics.

The Department of Scientific and Industrial Reseach has made grants as follow: to the Department of Physics, £16,680 for research under the direction of Prof. C. F. Powell for experiments in cosmic radiation with balloons, photon-induced cascades at different altitudes, and time variations in the primary cosmic radiation over Great Britain; £35,084 to the Department of Psychology, for research into the complexity of industrial tasks; to the Department of Geology, £5,000 for investigations of feldspars in the Connemara granite series and preferred orientation in rock-forming minerals; and £3,550 for investigations of the submarine geology of the continental shelf of the Western Approaches of the English Channel by Prof. F. Whittard; £3,850 to the Department of Physical and Inorganic Chemistry for equipment; and £2,880 to the Department of Zoology for investigations of the physiological basis of behaviour in protozoa. The National Coal Board has made a grant of £1,150 to the Department of Physical and Inorganic Chemistry for a study of carbon surfaces and surface complexes by infra-red spectroscopy, and £1,100 for a study of reactivity of carbon surfaces with oxygen. A grant not exceeding £1,150 has been made by the Ministry of Supply to the Department of Electrical Engineering for research on noise generation in inductors with ferromagnetic cores (continuation of grant).

London

Dr. P. B. D. DE LA MARE (New Zealand), reader in chemistry at University College, has been appointed to the University chair of chemistry tenable at Bedford College from October 1, 1960. The title of professor of experimental physics in the University of London has been conferred on Dr. F. C. Champion (Cambridge), in respect of his post at King's College. The following readerships are announced: Dr. D. R. Arthur (Wales), in zoology, in respect of his post at King's College; Dr. J. B. Hasted (Oxford), in physics, in respect of his post at University College.

Announcements

THE Physical Society has announced a conference on "Electron–Phonon Interaction in Solids", to be held in the Townsend Lecture Theatre, Clarendon Laboratory, Oxford, during December 17–18. Further information can be obtained from Miss E. Miles, Physical Society, 1 Lowther Gardens, Prince Consort Road, London, S.W.7.

THE Royal Institution has announced the one hundred and thirtieth course of six Christmas lectures adapted to a juvenile auditory. The lectures, entitled "The Release and Use of Atomic Energy", will be given by Dr. T. E. Allibone on December 29 and 31, 1959, and January 2, 5, 7 and 9, 1960. Further information can be obtained from the Royal Institution, 21 Albemarle Street, London, W.1.

THE Home Universities Conference, 1959, will be held in the Senate House of the University of London during December 11 and 12. The topics for discussion are: "University Co-operation in the Commonwealth"; "University Libraries"; and "Administrative Problems in Expanding Universities".

THE Institution of Mechanical Engineers is arranging an informal discussion on the Computer in Production, to be held on March 21 and 22. The purpose of the meeting is twofold: to introduce chartered mechanical engineers and managers to the latest techniques involving the application of computers in production; and to provide a forum for managers, engineers and users to present their views to manufacturers of computers. Visits to computer centres and to installations where computers are being used on production problems will be arranged. Further particulars can be obtained from the Secretary, Institution of Mechanical Engineers, 1 Birdcage Walk, London, S.W.1.

ERRATA. Prof. Daniel I. Arnon has pointed out the following mistakes which were overlooked in proof-reading in his article entitled "Conversion of Light into Chemical Energy in Photosynthesis" in *Nature* of July 4, p. 10: p. 11, col. 2, last word in the first paragraph should read "phosphoglycerate"; p. 11, equation 3, right-hand side, should be "4 Fe^{2+} . . ."; p. 17, col. 1, equation 7, left-hand side, should be ". . . $2(Chlp)^{+}$ . . .".

INSTITUTE OF PERSONNEL MANAGEMENT

WITH an attendance of some eight hundred members representing central and local government departments, industrial organizations, universities and other educational institutions from Britain and overseas, the annual conference of the Institute of Personnel Management took place at Harrogate during October 9–11. The theme was personnel management and technological change; and the conference was opened by C. H. Broadhead, a director of the Imperial Tobacco Co., Ltd., who carried out a wide-ranging survey of the growth, development and possible extension of personnel management in British industry. In this review, Broadhead emphasized the continuing need for recognizing the employee as an individual in his own right, and for the specialist personnel managers constantly to act so that general management never forgets its personnel function when there is the risk of subordinating the individual to the organization.

Then followed five sectional meetings during one of which comparisons were made between the progress of technological education in the United States, the U.S.S.R. and Great Britain. From his investigations Dr. G. Tolley, research manager of Allied Iron Founders, Ltd., suggested that there is every indication that the demand for trained engineers in Russia is now being satisfied by numbers currently graduating from full-time courses; it is probable that attention will now be concentrated upon the fulfilment of other objectives, for example, education in special technologies, such as in the chemical process industries. In addition, the existing facilities for training technicians and skilled workers, already organized on a vast scale, may be increased and supplemented. Although a great deal is known about the content of scientific and technological courses given in Russian educational institutions, little is known about Russian education for management and labour relations, and the key to the development of Russian society may lie here, rather than in the growth of science or technology as such. "Already, however, a largely Victorian society is rumbling rapidly on the move, and we would do well to remember that the huge reservoir of highly educated persons now being created in Russia will probably be capable of demanding something more than heavy industry."

At another meeting Miss J. Woodward and T. Lupton, of the University of Manchester, discussed the contribution of industrial sociology to management education. In the development of management theory Miss Woodward indicated that the early period was dominated by pioneers who sought to establish management theory on scientific foundations and to formulate laws which would describe phenomena and explain the relationship between cause and effect in an industrial situation. Classical management theory is based on the philosophy of the one best way, and is deterministic in that it assumes that this one best way can be found by suitable study and research. In the past twenty years a move away from determinism has taken place. It is now generally accepted that similar administrative expedients can, and often do, produce considerable variations in results. In the narrower field of the planning and control of manufacturing operations, tools of operational research are being used to isolate the variables linked with these variations. In the wider field of administrative behaviour, the move away from determinism has had less satisfactory results. There is a tendency to fall back on phraseology and proverbs and to assume that all variations are dependent solely on personality differences. The essential step in the establishment of a scientifically valid theory of management is the isolation and understanding of the variables in industrial situations. The most important facet of the investigations made by Miss Woodward in south-east Essex was not its challenge to classical management theory but its attempt to isolate one major variable in industrial situations from that of the actual techniques of production and to link this variable with observed variations in results. The information collected suggested that the techniques of a particular system of production impose demands on the human beings involved which limit and control their behaviour.

Studies of the behaviour of workers by Lupton and his colleagues have led to conclusions similar to those reached by Woodward in her studies of management. The behaviour of workers is influenced by such factors as the productive methods employed, the related administrative structures of the firm, and market factors. In the past five years six detailed investigations of the behaviour of workers, using participant observation as the main research technique, have been carried out. In only one of these, carried out among male engineering workers, was there behaviour resembling that found in the Bank Wiring Room of the Hawthorne experiments, that is to say, group controls over the level of output and earnings. In some workshops evidence was found of individualism and competitive striving as the normal mode of behaviour, while in others there was an absence of a clearly defined code of conduct relating to output and earnings. Further research, taking fuller account of other factors, may lead to a situational theory of workers' behaviour which challenges many of the naïve sociological and psychological generalizations which pass as 'principles of human relations'.

In a discussion on government policy and company pension schemes for manual workers, V. R. Jackson, head of the Pensions Department, Unilever, Ltd., argued that employers would be unwise to 'contract out' of the graduated part of the State scheme which comes into force in April 1961. Against this, H. A. R. Barnett, a consulting actuary, provided many reasons why employers should seize the opportunity to contract out.

The effect of equal pay on employment policies was examined by R. E. Griffiths, secretary to the Local Authorities' Conditions of Service Advisory Board. During the past five years equal pay has been introduced in the public services—including the Civil Service, the Local Government Service (including

teachers), the nationalized industries, and the Health Service. Progress to man's rate of pay is, by instalments, to be completed in 1961.

From observations of the workings of equal pay, Jackson has concluded that, where there is freedom of choice, equal pay will encourage the appointment and promotion of men in preference to women; equal pay may mean less security for women in times of recession; where women form a substantial proportion of an employed group, as in the basic teaching grade, equal pay-rates will tend to be lower than for a solely male group, and scales may be longer. This will tend to drive men out of the group; the increase in the inequity of living standards between family men and single women without dependants receiving equal pay will probably necessitate some form of additional family allowances; and, finally, equal pay raises costs, and may force reductions in the numbers employed.

The concluding session was concerned with the interaction of operational research and personnel management. The organization and mode of working of an operational research department was described by Stafford Beer, head of the Department of Operational Research and Cybernetics, United Steel Companies, Ltd.

Operational research involves the application of the methods of science to operations, whether military, industrial economic, or of any other kind. It has to consider systems of men and machines, materials and money; it is concerned with the regulation of these systems, with their decision processes, with their operation for maximum profit or minimum wastage or optimum use of resources. Operational research deals with the strategy of operations: not from general concepts of what management 'ought' to be like, but from the scientific principles of quantification, of inference, of experimental and analytical technique.

Beer suggested that operational research was relevant to personnel management for three reasons. First, the task of managing operational research itself has been little considered by those responsible for personnel management. Most big companies in most industries employ operational research workers. These departments have to be organized, and their organization poses very difficult and unusual problems. The departments have to be fitted into the existing organization of the parent company or institution on a routine basis, and this also raises difficult issues. The growth of this activity has been rapid: it has led to an extremely intractable education and training problem in which few educationists have taken an interest. Staff are still particularly hard to find, and their suitability hard to assess. By informing themselves about the topic of operational research, about its capabilities, its organization and its staff, personnel managers would put themselves in a position to help both their companies and the operational research movement to achieve a properly organized and integrated activity.

Secondly, an operational research discussion of its own organization is of possible interest to personnel managers in another way. Organization is itself a typical subject of operational research study. In discussing the organization appropriate for operational research, points of interest for other kinds of organization may well be uncovered. The third point of relevance for personnel managers is the most important. Operational research can certainly be applied to personnel problems themselves. Personnel management could well commission studies in its own field of problems, when operational research would be found capable of handling systems of exclusively human components. A good demonstration of it in practice was given by L. T. Wilkins and Herbert Mannheim in their scientific attack on operational problems of the Borstal system. This has been hailed as the greatest advance in penology for twenty-five years.

The application of operational research to the understanding of human beings was considered by Prof. R. W. Revans, of the Department of Industrial Administration, University of Manchester. The human problems of industry are not so much with individuals as with groups. There might be profit in trying to understand the nature of authority, the ways it is exercised and the effects that it produces. The highly complex relationships perceived as authority, namely, the willingness of one man to do what another man wants him to do, may change their nature simply by being discussed. To that extent these problems do not have the objectivity that is often regarded as a necessary attribute of scientific material. There is no evidence, for example, to suggest that if one hundred men sit down and talk about the law of gravity for a week, the nature of the law is in any way changed. But if one hundred men sit down and talk for a week about a strike in a motor factory, it is likely to make the strike very much worse. There is a difference between the study of the social problems of industry and the study of the problems of classical science, but it does not deny the validity of observational methods in the study of society.

A particular study of authority arises out of some work that Revans and his colleagues were invited to do at hospitals in the region of Manchester. The immediate and urgent problem was—and still is—that there are nothing like enough nurses in our hospitals and there seems little promise of obtaining substantially more.

An Indian student spent two years laboriously tracing the records of more than four thousand hospital employees in five comparable hospitals in Lancashire, and in 1958 brought out the first conclusions. Srivastava showed that, first, the voluntary wastage of nursing students during their three years of training differed significantly from one hospital to another and that, secondly, the hospitals with high voluntary wastage-rates among students had high wastage-rates among other members of staff, such as cleaners, auxiliary nurses, ward sisters and even the matrons themselves. All popular explanations, such as early marriage, the attractions of better paid jobs in the same town, even the decay of modern youth to-day, were shown to be illusory. There appears to be about every hospital some factor of cohesion which determines whether anybody in its service will want to stay there or not, and this cohesion (or lack of it) extends up to the matron and down to the most junior nurses.

It appeared that this factor of cohesion might have other manifestations. If the staff were prepared to stay at one hospital twice as long on average as at another, one might expect this to be reflected in some effect upon the patients. Srivastava found that the order in which the hospitals were placed for their ability to retain their student nurses was precisely the same as the order in which they were placed for the 'length of patient stay'. Srivastava chose cases which were, between hospitals, most directly

comparable. For this he chose cases of appendicitis. During the year 1958, considerably more than a thousand people were operated upon for this complaint in five hospitals; a perfect fit was found between the percentage of student wastage and the average length of patient stay. Average wastage ranged from 38 per cent in the hospital best able to retain its students to 65 per cent in the worst; the mean length of stay for appendicitis ranged from 7·5 days to 11·9. A significant concordance between all staff wastage and all clinical conditions examined was also found. Altogether, more than 5,000 cases of appendicitis, hernia, colectomy, partial gastrectomy, cystoscopy, asthma, bronchitis and pneumonia and head injuries have been examined; making allowance for differences between hospitals that have convalescent homes and those that have not, the hospital that can keep its staff longer can also deal with its patients more quickly.

This new law reinforces confidence in the organic concept of the hospital; it suggests that staff and patients seem to be influenced to the same extent. The source of this influence must be sought in the wards themselves. It has already been found that, in the hospitals with the high wastage rates, the student nurses particularly, but other nursing staff as well, complain of a general uncertainty about their work; their instructions often appear to be haphazard and it seems difficult to get decisions out of their committees. "A student nurse of 19 may not be afraid of hard work, does not complain about her money or the plainness of her diet, but is terrified of being put into situations which she does not understand because they have not been explained." A feeling of helpless ignorance is more corrosive of morale than the fear of any official rebuke which such ignorance may bring down. The girl who becomes a nurse has enough concern for her work to fortify her against the terrors of authority as such. But if she feels that she cannot carry her responsibility to her patients because she does not know what to do, she will in the end withdraw from an impossible situation, very often with regret.

Revans also drew upon later investigations to examine whether or not the character of the individual hospital is tied up with resistances to the friendly exchange of views and arrival at decisions. He suggested that this inquiry into the nature of authority will lead to new fields of inquiry and understanding.

ALKALI ION DETERMINATIONS BY MEANS OF GLASS ELECTRODES

THE possibility of measuring alkali ion concentrations in aqueous solutions by means of an electrode potential offers many advantages over other physical methods such as the flame photometer. Since the publication in 1957 by Eisenman, Rudin and Casby[1] on the sensitivity to sodium and potassium ions of glass electrodes made from sodium aluminosilicate glasses, interest in the subject has been growing rapidly and a Research Colloquium was held in the Department of Glass Technology, University of Sheffield, on May 6 to discuss the theory of the electrodes and some possible fields of application.

Dr. G. Mattock (Electronic Instruments, Ltd.) reminded those present that the development of glasses responsive to alkali metal ions has always been a possibility from the early recognition in studies on *p*H of glasses showing 'alkaline error'. In broad terms, the first requirement is to develop a glass which has a large alkali metal ion error. There seems to be no fundamental difference between glasses functioning for *p*H and those for alkali metal ions: all electrode glasses show some sensitivity both to hydrogen and to alkali metal ions, even, apparently, to other singly charged cations such as ammonium. The chief problem in devising compositions is the achievement of selectivity to a given ion in the presence of others.

Lengyel and Blum[2], Nikolskii and his co-workers[3], and Eisenman, Rudin and Casby[1] have all shown the importance of aluminium and/or boron in promoting alkali metal response when substituted for silica in a glass. The last-named workers have carried out a very exhaustive study on the $Na_2O - Al_2O_3 - SiO_2$ ternary system and have worked out extensive theories to cover the whole field of specific interactions at surfaces[4]. The fundamental importance of ion-exchange is accepted, the theory considering the factors affecting electrostatic attraction between ions in solution and the glass surface. The relative selectivity of glass response to different cations is qualitatively accounted for in terms of the competing effects of negative fields due to residual charges on oxygen ions (caused by the isomorphic introduction of trivalent aluminium or boron into the tetravalent silicon framework) and the hydrating tendencies of the bare cations. At low negative field-strengths on the lattice the ability to dehydrate a cation is weak, so that the order of selectivity (considered to depend on the effective radii of the ions) is related to the order of size of hydrated radii. At high field-strengths, this order can be reversed; and with intermediate fields, intermediate orders can be obtained—but significantly only eleven are theoretically predictable and only eleven have been found. These orders are:

(1) Cs, Rb, K, Na, Li	(7) K, Na, Rb, Li, Cs
(2) Rb, Cs, K, Na, Li	(8) Na, K, Rb, Li, Cs
(3) Rb, K, Cs, Na, Li	(9) Na, K, Li, Rb, Cs
(4) K, Rb, Cs, Na, Li	(10) Na, Li, K, Rb, Cs
(5) K, Rb, Na, Cs, Li	(11) Li, Na, K, Rb, Cs
(6) K, Rb, Na, Li, Cs	

Eisenman and Rudin are shortly to publish a full description of their treatment.

Dr. Matlock had been principally concerned up to now with studying the inductive effects of different polarizing substituents on the selectivity properties of glasses, and with obtaining easily workable glasses; a disadvantage of the original compositions of Eisenman, Rudin and Casby having been their extremely high softening points, making them difficult to fabricate into electrodes. Collaboration with Dr. Eisenman has now enabled development to be made on the basis of the Eisenman and Rudin theory, giving both ease of workability and improved selectivity. Glasses responsive to potassium have

also been made. There seems to be a wide field of application in chemical analysis, in salinity measurements on waters and soils and in various industrial fields, as well as in biochemical and medical spheres.

Dr. W. Bartley, Mr. A. Renshaw and Miss B. M. Notton (Cell Metabolism Research Unit, University of Oxford) described tests carried out on experimental electrodes of aluminosilicate glasses prepared in the Department of Glass Technology, Sheffield. Sodium aluminosilicate glasses similar to those recommended by Eisenman for sodium ion measurements were compared with sodium alumino-borosilicate glasses, in which some alumina and/or silica had been replaced by boric oxide in order to improve the technological properties.

Electrodes were filled with an acetate buffer (0·15 *M* sodium acetate + 0·1 *M* hydrochloric acid *p*H 4·8) in which dipped a silver – silver chloride electrode. The reference electrode was of the Beckmann 'fibre' type, the potentials were read on a 'Vibron' electrometer (Electronic Instruments, Ltd.) and recorded on a 'Record' pen recorder. The potentials were measured of solutions of sodium, potassium and lithium ions and hydrochloric acid ranging from *M* to 10^{-5} *M* and of mixtures of sodium and potassium ions in the same range. All solutions except hydrochloric acid were buffered to *p*H 10 by the addition of *tris*(hydroxymethyl)aminomethane. The test solutions were left in contact with the electrode until a stable voltage was recorded and the time taken for this to occur was noted.

In Table 1 the rates of change of voltage with concentration are given for each of the four cations with each glass. In general, the slopes with sodium ions were similar to those with hydrogen ions, ranging between 50 and 60 mV. for each tenfold change of concentration. The slopes with potassium and lithium ions were some half to three-quarters of that with sodium and varied considerably with glass composition. It can be seen that the introduction of boric oxide has caused no systematic change in the alkali ion sensitivities of the sodium aluminosilicate glasses.

Table 2 shows the voltages developed in molar solutions of the ions. The values for *M* H^+ and *M* Na^+ are considerably higher than the values for *M* K^+ and *M* Li^+. Again it is difficult to observe a consistent trend on the introduction of boric oxide.

Table 1. Response of Various Glass Electrodes to Changes in Ion Concentration

Glass	Average slope (mV./*p* unit) with: Na^+	K^+	Li^+	H^+
NAS 11–18*	52	34	22	52
NAS 13–20	50	36	40	56
NAS 12½–12½	50	40	50	60
NABS 1/1/1/4†	58	30	50	52
NABS 1/1/½/5	52	34	46	49
NABS 1/½/½/6	55	42	34	57

Table 2. Voltage given by Various Glass Electrodes with the Same Concentration of Different Ions

Glass	Voltages (mV.) with molar concentration of: Na^+	K^+	Li^+	H^+
NAS 11–18*	+ 93	− 102	− 26	+ 138
NAS 13–20	+ 100	− 47	− 8	+ 131
NAS 12½–12½	+ 100	− 36	+ 10	+ 185
NABS 1/1/1/4†	+ 91	− 90	− 16	+ 162
NABS 1/1/½/5	+ 96	− 56	− 18	+ 129
NABS 1/½/½/6	+ 88	− 32	− 48	+ 100

* Molecular per cent of sodium monoxide and alumina respectively.
† Molecular ratios of sodium monoxide/alumina/boron trioxide/silica. Thus it may be noted that sodium aluminosilicate 12·5/12·5 is equivalent to sodium alumino-borosilicate 1/1/0/6.

The behaviour of the electrodes was investigated under the important practical conditions occurring when two alkali ion species were present simultaneously. It was anticipated that potassium, for example, would interfere with the sodium response of the electrodes when the voltages produced by the two ions separately were about the same. Thus *M* potassium chloride would be expected to interfere with all solutions of sodium chloride below 0·001 *M*. In the sodium aluminosilicate series of glasses this was true, but in the sodium alumino-borosilicate series interference of the potassium ion already occurred at 0·1 *M* sodium chloride. In general, it was not found possible to predict the response of an electrode to a solution containing two ion species, on the evidence of its response to each separate ion. Thus the introduction of boric oxide into the sodium aluminosilicate glasses caused a great deterioration of specificity not revealed by the data of Tables 1 and 2.

The time taken for the electrodes to register a stable voltage was also measured. It varied with the composition of the glass and also with the composition of the ion mixture; but, in general, it was increased by the introduction of boric oxide. Thus, the sodium aluminosilicate glasses reached equilibrium in a matter of a few minutes while the sodium alumino-borosilicate glasses took some hours to reach equilibrium. All electrodes responded fastest at *p*Na 1 and most slowly at *p*Na 2 or 3. In the presence of potassium ions the equilibration time was increased but the electrodes still reached equilibration fastest at *p*Na ½ and slowest at *p*Na 2 or 3.

Of the glasses tested the most useful and least subject to various interferences were the sodium aluminosilicate series; of these sodium aluminosilicate 11–18 was the best. Measurements were made of the sodium content of standard solutions of various sodium salts with a sodium aluminosilicate 11–18 electrode calibrated against sodium chloride. With this system, although the measured and standard solutions usually agreed to within 10 per cent, divergencies of up to 40 per cent could occur. However, differences of less than 1 per cent in the sodium content of two solutions of a given salt could be detected easily by the use of the electrodes, although the precise concentrations of either solution could not be measured accurately.

It was concluded that the electrodes used could not be relied upon for accurate measurements, but were capable of giving a sensitive indication of changes in ion concentrations.

Dr. J. S. Stanley (J. A. Radley's Industrial Research and Development Laboratories, Reading) described the application of glass electrodes responsive to sodium to the study of micelle formation in solutions of paraffin chain salts and dyestuffs. Aggregation is easy to detect from the fall in the activity coefficient of the sodium ion, which occurs above the critical micelle concentration. The critical micelle concentrations of sodium laurate, sodium lauryl sulphate and dioctyl sodium sulphosuccinate determined by this method agreed well with previous determinations by other methods, and aggregation in several dyestuff solutions has been detected.

The method is particularly useful for determining the association of the counter ion with the micelle, since the electrode determines sodium ion activities directly. Dr. Stanley said that, so far as he could

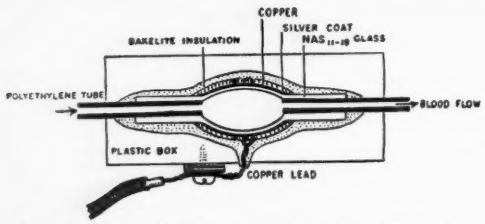

Fig. 1. Diagrammatic section through a tubular type of glass-to-silver sodium electrode. Measurements are approximately 0·5 cm. × 2·0 cm.

ascertain, this was the first time that glass electrodes responsive to sodium had been applied to problems outside the biochemical field.

Dr. J. A. M. Hinke (American Life Insurance Medical Research Fellow, Department of Biophysics, University College, London) discussed the application of glass electrodes responsive to alkali ions in experimental biology and medicine. He reviewed the design and performance of a tubular type sodium electrode for measuring plasma sodium of an anæsthetized animal (Fig. 1)[5]. A thin bulb was blown in the middle of a tube of Eisenman sodium aluminosilicate 11–18 glass and electrical contact was made to the outer surface by a coating of silver. Potential changes were amplified with a Cary vibrating reed electrometer and recorded on a Grass model 5 polygraph. The electrometer provided enough gain to detect changes of 1 milliequivalents sodium/litre in a total of 140 milliequivalents/litre. Such performance indicates two possible advantages of these electrodes over the alternative method of flame photometry for alkali ion determinations, namely, the detection of less than 1 per cent change in sodium concentration and the possibility of continuous recording.

Evidence was presented that changes in the flow-rate influence the response of the tubular electrode, but it was shown that this limitation can be minimized by a suitable design of the electrode shape.

A second application of glass electrodes has been made on a micro scale (see *Nature*, October 17, p. 1257), the glass for which was supplied by Friedman (University of British Columbia) and behaved similarly to Eisenman's sodium aluminosilicate 11–18 and 27–8 glass. Table 3 indicates the performance of a microelectrode sensitive to sodium over the physiological molar concentration range of sodium and potassium at *p*H 8·12. Other experiments were cited which indicated that hydrogen ion interfered significantly below *p*H 6·5 but that affects of *p*H could be ignored between *p*H 7 and 8. Over this biological range of sodium, potassium and hydrogen ions, the electrodes behave according to Eisenman's modified empirical formula[6]:

$$E = E^{0} + \frac{RT}{F} \ln(a_{Na} + k_{NaK} a_{K})$$

where E is measured electromotive force, E^{0} is standard potential, a_{Na} and a_{K} are activities of sodium and potassium ions respectively and k_{NaK} is empirical constant for a given glass composition.

Table 3. Experimental Response (mV.) of Sodium Ion Sensitive Microelectrodes

All solutions buffered to *p*H 8·12 with 0·2 *N tris*(hydroxymethyl)-aminomethane and 0·1 *N* hydrochloric acid

	[K⁺] 0·016	[K⁺] 0·04	[K⁺] 0·08	[K⁺] 0·12	[K⁺] 0·16
[Na⁺] 0·016	− 53·5	− 53·5	− 53·5	− 53·5	− 53·5
[Na⁺] 0·04	− 31·0	− 31·0	− 31·0	− 31·0	− 31·0
[Na⁺] 0·08	− 13·5	− 13·0	− 13·0	− 13·5	− 13·5
[Na⁺] 0·12	− 3·5	− 3·5	− 3·5	− 4·0	− 4·0
[Na⁺] 0·16	+ 3·5	+ 3·5	+ 3·5	+ 3·5	+ 3·0

Given standardized sodium and potassium selective electrodes, the potential of each can be measured in an electrolyte system and the equation can be solved for A_{Na} and A_{K} simultaneously. This has been done inside muscle cells of crab and lobster using the microelectrodes as already described in *Nature* (*loc. cit.*).

The discussion which followed emphasized the practical advantages of determining alkali ions by electrodes rather than by flame photometry; these electrodes can give greater sensitivity, they can be used non-destructively with continuous recording and they give a direct determination of activities, instead of total concentration, which may be of great importance in biological or colloidal systems. The theory of action of the electrodes was discussed at some length and the need for a fuller investigation of the effects of varying the glass composition was emphasized.

J. O. Isard

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POLARIZATION OF LIGHT FROM NOCTILUCENT CLOUDS

By GEORG WITT
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MEASUREMENTS of the polarization of light from noctilucent clouds have recently been made in Sweden. A commercial 35-mm. stereo-camera body was fitted with a pair of *f*/1·65, 50 mm. Petzval projection lenses. Rotatable 'Polaroid' filters, geared to remain mutually perpendicular, were mounted in front of the lenses. Sliding Wratten gelatine filters were used to select the desired range of wave-lengths. The image sizes were 22 mm. × 22 mm., 1 mm. corresponding to approximately 1° in the field of view.

This equipment was set up for operation at Torsta (lat. 63·3° N., long. 14·6° E.) during July and August 1958. The station has been used for noctilucent cloud observations since 1955 (see refs. 1 and 2). A brilliant display, in good visibility conditions, occurred on August 10–11, 1958, and a sequence of polarization measurements was carried out as follows.

The camera was directed at a bright feature in the cloud system. Knowing the position of the Sun, the 'Polaroid' filters were orientated to be parallel and perpendicular to the plane containing the Sun, the cloud, and the observer. Two exposures were made in quick succession with two different colour filters, the first centred on 4900 A., the second on 6100 A. (effective centres on the emulsion used). Exposures varied from 5 to 60 sec. on Kodak Plus-*X* emulsion. The procedure was repeated for different cloud features covering as wide a range as possible in solar elongation. Neutral step-density filters were photographed on the same emulsion, and developed with the cloud records, to provide calibration for subsequent analysis by photodensitometry. Neutral step-density filters were photographed on the same emulsion, and developed with the cloud records, to provide calibration for subsequent analysis by photodensitometry. The brightness of the features used was at least four times greater than that of surrounding minima, which must include the twilight foreground light. Thus no correction for this component was considered necessary.

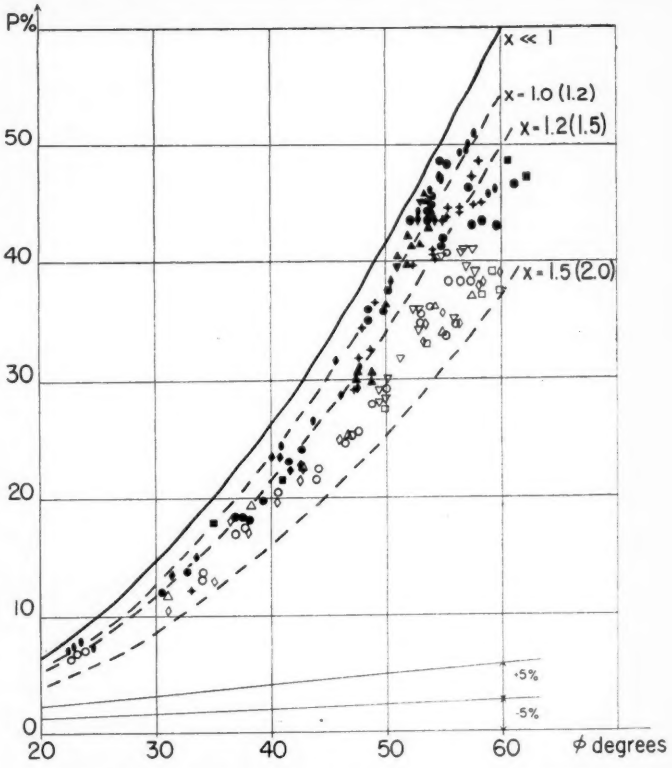

Fig. 1. Degree of polarization as a function of scattering angle. Open symbols refer to the blue wave-length (λ = 4900 A.), and filled symbols to the red (λ = 6100 A.). The magnitude of an allowed scatter of 5 per cent is shown by straight lines at the bottom

The interpretation of these results is based upon the Mie theory of scattering of light by small particles (see Van de Hulst[3]). If I_1 and I_2 are the intensities of the components of the scattered light polarized perpendicular and parallel to the plane of incidence and scattering respectively, then the degree of polarization of the scattered light is defined as $P = (I_1 - I_2)/(I_1 + I_2)$. In this application, the cloud is assumed to be illuminated by natural light from the Sun. The theory predicts that the polarization and intensity of scattered light are functions of the scattering angle φ, the size parameter $x2 = \pi r/\lambda$, where r is the radius of the particle and λ the optical wave-length, and of nx, where n is the refractive index of the particle. At low elevation angles, selective extinction in the atmosphere is serious, but there is no reason for expecting the polarization to be significantly affected. This is an important advantage of polarization over spectral observations.

In Fig. 1 the horizontal scale is the computed scattering angle and the vertical scale is the degree of polarization. The limiting case of Rayleigh scattering is shown by the solid curve. The broken lines show the predicted polarization for particles with x = 1·0, 1·2 and 1·5, as indicated, if the refractive index n is 1·55. For n = 1·33 (water) the values of x are those given in brackets. Measured polarization values are shown by the symbols, which are of different shape for different pairs of exposures. Seven blue and eleven red exposures were made on various parts of the cloud over a period of 3 hr. Open symbols refer to the blue wave-length, and filled symbols to the red.

Points to note are as follows. The observed behaviour is of the kind predicted by the theory for a rather limited range of particle sizes, and the same range of sizes accounts for the results on the two separate wave-lengths. Systematic errors of analysis could scarcely be invoked to account for this regularity of behaviour. Thus a value of the radius r = (1·0 ± 0·1) 10^{-5} cm. (n = 1·55) accounts for the results. There is evidently not an appreciable proportion of particles of larger than 2 × 10^{-5} cm. radius; one cannot say if there are smaller particles present. There is no evidence, so far, of any change in the effective radius during the progress of the display, or from place to place in the cloud.

Further polarization measurements are being made. Stereophotogrammetric results obtained at Torsta on the same cloud have shown that the material of the cloud moved in a south-westerly direction with a velocity of the order of 100 m./s., while a system of bright waves moved more slowly in the reverse direction. The effect has been demonstrated in a convincing manner by time-lapse films taken of these and subsequent clouds. It is the movement of the bright waves which has commonly been reported in the past[4].

I am indebted to Dr. and Mrs. F. Volz, and Mr. N. Wilhelm for valuable assistance. The astronomical institutions of Stockholm and Uppsala provided the facilities for microdensitometry.

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REDUCTIVE PHOTO-BLEACHING OF EOSINE

Primary Processes indicated by the Flash Technique

It is well known that a de-aerated solution of eosine in ethanol when irradiated undergoes reductive bleaching. Imamura and Koizumi[1] concluded, from the kinetic studies of this reaction, that a semiquinone radical with a life-time not less than 10^{-3} sec. is formed from the triplet state of dye and ethanol.

The primary processes of this photo-bleaching have now been studied by the flash technique, and in addition to semiquinone, another intermediate with a life-time of the order of 10^{-3} sec. was found spectroscopically. A photolysis flash lamp and a spectro-flash of 'half-width' approximately 60 and less than 20 μsec. respectively were used. The results obtained may be summarized as follows.

At 0·6 msec. after the start of the photolysis flash, the main absorption of eosine decreased to 60 per cent of that of the initial solution and a transient increase of absorbance was observed in the region from 350 to 480 mμ. Subtracting from this the absorbance due to the remaining eosine, the quantity of which was evaluated from the depression of the absorbance in the region of wave-lengths longer than 480 mμ, the spectra of the transient species were obtained (see Fig. 1).

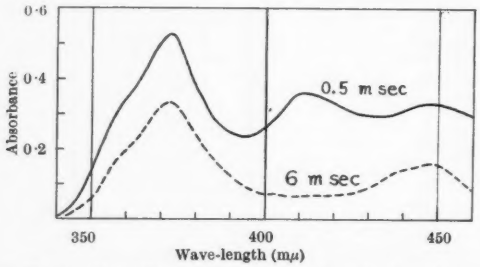

Fig. 1. Absorption spectra of transient species in eosine

Three peaks occur, at 373, 410 and about 450 mμ. After 5 msec., the peaks at 373 and 450 mμ (*A* band) still maintained considerable intensity, whereas 410 mμ (*B* band) declined appreciably. After several minutes, the former band also completely vanished and the eosine spectrum was recovered, corresponding to 88 per cent of that of the original solution. The 12 per cent loss is attributable, without doubt, to permanent bleaching.

In the presence of air, neither transient spectral change nor permanent bleaching was observed. The de-aerated aqueous solution of eosine also showed no such transient spectral change.

These results imply that two transient species are formed in the initial stage of the reductive photo-bleaching of eosine, and that at a certain early stage after flashing, most of the dye molecules are converted into such intermediate species with life-times not less than about 10^{-3} sec.

There is little doubt that the *A* band is due to the semiquinone radical of eosine. As regards the *B* band, it is certain that this is not due to a triplet state, because no similar band is observed in an aqueous solution in which the triplet state is not deactivated so efficiently as in ethanol solution. Hence the *B* band must be attributed to some unknown molecular species which perhaps precedes semiquinone; it would be reasonable to attribute this to a certain kind of molecular complex between excited dye and ethanol.

The primary processes of the photo-bleaching may be pictured as follows:

$$
\begin{array}{lllll}
D + h\nu & \rightarrow & D^* & & (1)\\
D^* & \rightarrow & D & + h\nu' & (2)\\
D^* & \rightarrow & D^t & & (3)\\
D^t + RH & \rightarrow & X & & (4)\\
X & \rightarrow & D + RH & & (5)\\
X & \rightarrow & DH^{\cdot} + R^{\cdot} & & (6)
\end{array}
$$

where D and RH represent dye and ethanol, and X is the molecular complex suggested above; the other symbols have the usual meaning. This scheme accounts for all the experimental results, including those formerly obtained from kinetic studies. Only two of them will be taken up here. (*a*) The observation that only 12 per cent is permanently bleached in spite of the fact that almost all the dye molecules are converted to intermediates with life-times not less than about 10^{-3} sec. is due to process (5), which plausibly proceeds faster than (6). (*b*) The fact already established that the reductive photo-bleaching is completely inhibited by the presence of a very small quantity of oxygen is interpreted as due to X being deactivated by oxygen by the process $X + O_2 \rightarrow D + RH + O_2$. Imamura and Koizumi's scheme that semiquinone is destroyed by oxygen is not in agreement with the fact that the *B* band is not observed in the aerated ethanol solution.

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Effect of Added Substances on the Rate of Reductive Photo-bleaching

The alcoholic solution of eosine, when de-aerated and irradiated by visible light, undergoes photo-bleaching which proceeds via the formation of a semiquinone of the dye[1]. The effect of the added substances on the rate of bleaching has now been examined, and it is found that some substances accelerate the rate appreciably and in a very specific manner.

A sample degassed carefully by repeated vacuum distillation was illuminated by light from a tungsten projection lamp, transmitted through a filter cutting off wave-lengths less than 450 mμ.

Since the absorption spectra of the irradiated solution, in all cases, gradually declined, keeping the same shape as that of the original solution, the rate of bleaching was traced simply by measuring the absorption spectra at suitable time intervals with a Hitachi spectrophotometer *EP U-2A*.

The rate of bleaching can always be expressed by the formula:

$$-\frac{dc}{dt} = \frac{k}{d} I_0 \left\{1 - \exp(-\alpha c d)\right\} \times 10^3 \quad (1)$$

where c is the concentration of dye in mole/litre, I_0 is the intensity of illuminating light (mole cm.$^{-2}$ sec.$^{-1}$), α is the effective molar absorption coefficient and d the thickness of the solution in centimetres.

Integration of (1) gives:

$$\ln(\exp \alpha c_0 d - 1) - \ln(\exp \alpha c d - 1) = 10^3 k I_0 \alpha t \quad (2)$$

By using the properly determined value of α, one can obtain k from the inclination of a linear plot of $\ln(\exp \alpha cd - 1)$ against t.

Typical results showing the effect of the added substances are summarized in Table 1 (temperature, 25° C.).

Table 1
RETARDING EFFECT

*Iso*pentane		*n*-Hexane	
Mole fraction	Relative k	Mole fraction	Relative k
0·334	0·20	0·309	0·59
0·143	0·43	0·130	0·67
0·0814	0·84	0·073	0·89
0·0529	0·85	0·047	1·01
0	1	0	1

Benzene		*Cyclo*octatetraene	
Mole fraction	Relative k	Mole fraction ($\times 10^3$)	Relative k
0·220	0·61	650	0
0·141	0·63	65	0·26
0·0681	0·69	32·2	0·42
0·0334	0·70	8·07	0·61
0	1	0	1

ACCELERATING EFFECT

n-Hexene		*Cyclo*hexene	
Mole fraction	Relative k	Mole fraction	Relative k
0·0181	3·62	0·0295	6·4
0·0119	2·69	0·0146	3·53
0·00593	1·99	0·00726	2·43
0·00296	1·74	0·00362	1·71
0	1	0	1

Since all these substances scarcely affect the absorption or fluorescence of eosine, the effect on the rate ought to be caused mainly by their interaction with the triplet state of the dye, or with any other species produced from it. If one assumes, as has usually been done, that the triplet state is deactivated by the retarding substances (Q-groups), according to the process $D^t + Q \rightarrow D + Q$, then the relation:

$$\frac{1}{k} = a + b[Q]/[RH] \qquad (3)$$

must hold approximately, where RH is ethanol.

This fits the experimental results. The effect of *n*-hexene and *cyclo*hexene is very specific in that the rate increases according to the relation (Fig. 1):

$$k = a' + b'[P] \qquad (4)$$

Assuming that these substances also attack the triplet state, then the above relation implies that the attack mainly results in the formation of semiquinone without any accompanying measurable deactivation process. This is rather curious in view of the behaviour of the Q group.

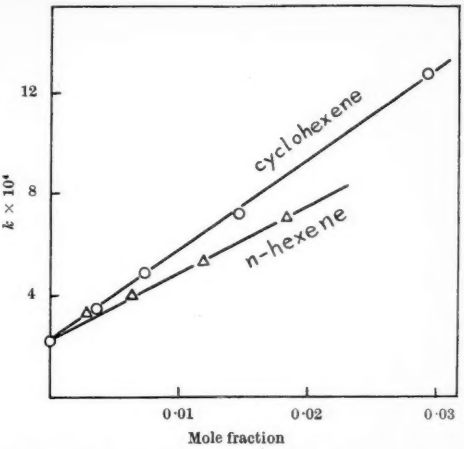

Fig. 1. Plot of k against the concentration of the added substances

Another possibility to be taken into consideration is that P attacks a complex between excited dye and ethanol, $(D\cdots H—R)$, since the complex formation has now been confirmed by the flash experiments (preceding communication).

If one assumes that the two substances of the P group attack $(D\cdots H—R)$ only in such a specific manner that R is detached from the latter, producing a more stable radical $PR\cdot$ and leaving behind $DH\cdot$, then the rate of bleaching would be increased and the relation (4) can be accounted for.

A definite conclusion must, however, await further work, especially by the flash technique, since the introduction of the complex has made it necessary to examine more carefully whether the added substances of the P group and also those of the Q group attack chiefly D^t or $(D\cdots H—R)$.

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CONTROL OF HATCHING IN THE PREDIAPAUSE EGGS OF *AEDES* MOSQUITOES

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EGGS of *Aedes* mosquitoes are usually laid above water-level, where they may remain viable for long periods while awaiting submergence. On flooding, however, they do not usually all hatch together, even though they may all be the same age. Indeed, hatching, even in continuously submerged eggs, may go on in small instalments over a period of many months. This erratic hatching-response is well known, and results in a temporal spread of newly hatched larvæ—an obvious advantage to

species which tend to utilize small collections of water subject to irregular changes in level and to sudden drying. To the experimental worker, however, wanting large numbers of larvæ of the same age, erratic hatching is a nuisance. Hence, much of the work that has been done on this subject has tended to ignore the biological significance of erratic hatching and has concentrated on a search for a stimulus that will make all eggs hatch together. Many such stimuli have been described over the past forty years, and the references are too numerous to include here; suffice it to say that most of these involve a lowering of the amount of dissolved oxygen in the water[1]. While it is obviously useful to know what can stimulate hatching in *Aedes* eggs, it is, perhaps, of greater biological interest to study the factors which inhibit it.

It has previously been shown that, shortly after reaching embryonic maturity, unhatched but submerged and apparently hatchable eggs of *Aedes* (*Stegomyia*) *aegypti* (L.) are overtaken by a state of genetically determined diapause, the depth of which varies with the passage of time, the manner of this variation with time itself varying from one egg to another[2,3]. This obligate diapause differs in certain respects from many other instances of diapause[4,5] and can be broken at any stage by the application of a maximal stimulus[3]. In Nature, however, it is probable that this strength of stimulus is never found, and hence only a few eggs hatch each time a stimulus occurs. Thus, hatching by small instalments is ensured, once diapause has been entered. The onset of diapause, however, does not always coincide with the attainment of embryonic maturity and a variable amount of hatching sometimes occurs before diapause has been reached. Indeed, prediapause hatching is usual in *A.* (*S.*) *africanus* Theo., *A.* (*S.*) *apicoargenteus* Theo., *A.* (*Finlaya*) *longipalpis* Grunb. and in some strains of *A. aegypti*[2].

In a strain of *A. aegypti*, colonized in Entebbe, Uganda, in 1958, from eggs sent from Ilobi, Nigeria, by Mr. J. P. T. Boorman, prediapause hatching in a proportion of submerged eggs occurs on days 3 and 4 after laying; entry to diapause of the remaining unhatched eggs occurs on day 5, after which no further hatching occurs in the absence of a hatching stimulus. The proportion which thus hatch during the prediapause state varies widely from one batch of eggs to another, all proportions having been recorded from 0 to 76 per cent. Faced with this extreme degree of variability, it is a remarkable fact that never do they all hatch during the prediapause period. It would appear that some mechanism comes into play which prevents unchecked hatching during this precarious period, thus ensuring that at least some eggs reach the diapause state. It was postulated, therefore, that the first eggs to hatch might make conditions less favourable for the remaining unhatched eggs.

In order to test this hypothesis it was first necessary to minimize variation by randomizing the eggs for parentage. This was easily achieved by setting up a special colony of about 500 males and 500 females, reinforced by about 50 newly emerged adults of each sex per week, and making daily collections of the eggs which had been laid communally on a single small piece of moist filter paper. When laying, the females do not deposit all, or even many, of the eggs in one place, but walk over the surface of the paper, stopping every few seconds to attach a single egg here and another there, and so on. It follows that, by the time egg-laying activity is finished, which in this species is restricted to the last two or three hours of the daylight period[6], a mass of eggs results, any portion of which will be of mixed parentage. It was taken as axiomatic that early-laid eggs usually reach embryonic maturity, and thus tend to hatch, before late-laid eggs, and the following account may be considered as an investigation into the possibility that hatching in early-laid eggs depresses hatching in late-laid eggs.

Eggs laid on filter paper on a single day were carefully washed and submerged in tap water on the day of laying. These are referred to as group *A* eggs. For ease of handling and subsequent counting, the day's lay was arbitrarily divided into ten sub-groups by cutting the paper at convenient places—each piece of paper bearing approximately the same number of eggs. Each piece was placed in a sterile glass tube containing 3 ml. tap water. 24 hr. later the next day's lay was similarly treated (group *B*), but the pieces of paper bearing the eggs were placed in the tubes of water already containing the group *A* eggs. Thus, each tube contained two pieces of paper bearing eggs, one (group *B*) being 24 hr. younger than the other (group *A*). Both groups were kept submerged for five days at a temperature of 29° C. and in constant light. The papers bearing the eggs were removed on day 5 (group *A* on one day, group *B* on the following day), and the hatched eggs counted under a binocular dissecting microscope. The papers were then placed in a medium containing a maximal stimulus for a period of 24 hr. in order to test for viability of the unhatched eggs[3]. The response was expressed as the number of eggs which hatched within five days, out of the total number of viable eggs present. The result of this initial trial (combining the ten sub-groups) supported the hypothesis that hatching in early-laid eggs depresses hatching in companion late-laid eggs; 51 per cent of group *A* eggs hatched (226/440), 26 per cent of group *B* eggs hatched (126/478). Nevertheless, with such variable material, it was decided to make a total of ten such trials, with ten sub-groups in each. The full results, including those of trial 1, are shown in Table 1, from which it will be seen that all the results are in the

Table 1. Hatching in Submerged Prediapause Eggs of *A. aegypti*. Influence of Hatching of Early-laid Eggs (group *A*) on Late-laid Eggs (group *B*), Both Groups Sharing the Same Container of Water

Trial No.	Interval in hours between laying *A* and *B*	Group *A* Proportion hatched by day 5	Per cent	Group *B* Proportion hatched by day 5	Per cent	χ^2 ($n=1$)	Significance at 1 per cent level + or −
1	24	226/440	51	126/478	26	60·587	+
2	,,	209/476	44	172/534	32	14·659	+
3	,,	302/538	56	134/480	28	82·488	+
4	,,	250/635	39	122/502	24	28·914	+
5	,,	502/874	57	235/748	31	110·069	+
6	,,	210/278	76	78/292	27	135·827	+
7	,,	371/1,006	37	184/568	32	3·198	−
8	,,	202/298	68	192/686	28	137·705	+
9	,,	485/1,002	48	378/1,187	32	62·379	+
10	,,	276/440	63	169/411	41	38·897	+
Total		3,033/5,987		1,790/5,886			
		51 per cent		30 per cent			

$\chi^2 = 504{\cdot}515$

$P < 0{\cdot}001$

Table 2. HATCHING IN SUBMERGED PREDIAPAUSE EGGS OF *A. aegypti*. INFLUENCE OF HATCHING OF EARLY-LAID EGGS (GROUP *A* TEST) ON LATE-LAID EGGS (GROUP *B* TEST), BOTH GROUPS SHARING THE SAME CONTAINER OF WATER. CONTROLS (GROUPS *A* AND *B*) EACH IN CONTAINERS BY THEMSELVES

Trial No.	Interval in hours between laying *A* and *B*	Group *A* Proportion hatched by day 5				χ^2 ($n = 1$)	Significance at 1 per cent level + or −	Group *B* Proportion hatched by day 5				χ^2 ($n = 1$)	Significance at 1 per cent level + or −
		Control		Test				Control		Test			
			Per cent		Per cent				Per cent		Per cent		
1	<2 >1	204/455	45	245/459	53	6·671	+	230/460	50	183/467	39	10·970	+
2	,,	273/529	52	268/577	47	2·952	−	239/492	49	209/477	44	2·028	−
3	24	274/543	51	258/472	55	1·786	−	220/579	38	163/533	31	6·758	+
4	,,	281/497	57	297/535	56	0·110	−	337/574	59	209/599	35	66·840	+
5	,,	235/476	49	270/472	57	5·842	−	223/453	49	189/384	49	0·056	−
6	,,	248/501	50	251/449	56	3·891	−	271/515	53	207/557	37	25·879	+
7	,,	270/572	47	263/526	50	0·858	−	242/563	43	173/598	29	24·939	+
8	,,	202/461	44	237/514	46	0·515	−	339/702	48	161/556	29	48·427	+
9	48	230/441	52	236/442	53	0·136	−	217/447	49	177/465	38	10·204	+
10	,,	272/548	50	267/609	44	3·890	−	365/718	51	145/546	27	75·959	+
Total		2,489/5,023		2,592/5,055				2,683/5,503		1,816/5,182			
		50 per cent		51 per cent				49 per cent		35 per cent			
		$\chi^2 = 2·995$						$\chi^2 = 205·813$					
		$0·05 < P < 0·10$						$P < 0·001$					

expected direction, the hatch in group *B* being significantly lower ($P < 0·001$) in every trial except trial 7.

While these results are consistent with the hypothesis, they can, of course, be interpreted in the reverse way: that the presence of group *B* eggs in some way enhanced the hatch in group *A*. Therefore, a more critical experiment was planned using separate controls for groups *A* and *B*. Eggs of group *A* were set up as test and control groups. Similarly, eggs of group *B* were set up as test and control groups, group *B* test eggs being added to the water already containing the group *A* test eggs. The response was expressed as the proportion of group *A* test eggs which hatched within five days compared with the proportion of group *A* control eggs which hatched in the same period, and the proportion of group *B* test eggs which hatched within five days compared with the proportion of group *B* control eggs which hatched in the same period. Again, ten such trials were made with ten sub-groups in each trial, but the interval between the laying of groups *A* and *B* was varied from between 1 and 2 hr. to 48 hr. The results are shown in Table 2, and fully support the interpretation of the preliminary ten trials.

These consistent and clear-cut results based on large numbers of eggs leave little doubt that the first eggs to hatch in some way influence the remaining unhatched prediapause eggs. Now, prediapause hatching is spread over a period of about 24 hr. on days 3–4, which is much longer than would be expected on the strength of the age differences in a mass of eggs laid in a single egg-laying period of 3 hr. While some of this scatter probably owes its origin to innate variation on the part of the eggs themselves, it is perhaps increased by an influence from the first eggs to hatch. It seems possible that as the number of early hatchers increases there is a progressive delay in hatching of the remaining eggs until further hatching is prevented by the onset of diapause on day 5. This preliminary interpretation receives some support from the results of a pilot trial. Eggs obtained during a single egg-laying period were divided into three groups, *A*, *B* and *C*. 24 hr. later 100 and 1,000 newly hatched larvæ were added to groups *B* and *C*, respectively. The proportion of eggs which hatched in the three groups by day 5 was as follows: group *A* (control with 0 larvæ) 362/678 (53 per cent); group *B* (100 larvæ) 323/673 (48 per cent); group *C* (1,000 larvæ) 270/721 (38 per cent). While these results are consistent with this view, and the differences are not only in the expected direction, but that between groups *B* and *C* is highly significant ($\chi^2 = 15·838$, $P < 0·001$), they would obviously have to be repeated many times before they could be accepted with confidence.

There is no information yet on whether the influence results indirectly from metabolic contamination of the medium, or directly from the action of some specific diffusible hormone. Preliminary trials have not shown any change in *p*H of the medium during this period, even when very large numbers of eggs are involved. Some very interesting work has recently been done on the changes in permeability of the chorion in *Aedes* eggs which occur during the transition from the prediapause to the diapause state[7,8], and it would be interesting to know if these changes are affected by the presence of recently hatched larvæ in the medium.

Contrary to these findings, some of the early work on egg-hatching in this genus indicated that the presence of larvæ may induce hatching in eggs kept in the same container[9,10]. It is important to note, however, that these former results were obtained with eggs in the diapause state; in a later paper evidence will be presented which indicates that hatching in diapause eggs may, in fact, induce hatching in companion diapause eggs by lowering the threshold of response to a given stimulus. Thus, whereas hatching in the prediapause state depresses further hatching, the reverse holds for diapause eggs.

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ROLE OF A DAGGER NEMATODE (*XIPHINEMA* SP.) IN OUTBREAKS OF PLANT DISEASES CAUSED BY ARABIS MOSAIC VIRUS

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FOR many years arabis mosaic virus was solely of academic interest, having been found by Smith and Markham[1] only in a single plant of *Arabis* sp. growing in an insect-proof glasshouse at Cambridge, England: its natural mode of spread was unknown. Recently, however, one of us (C. H. C., ref. 2) has shown by serological tests that raspberry yellow dwarf virus, a soil-borne virus described in 1958 by Harrison[3], is closely related to and should be regarded as a strain of arabis mosaic virus. Hence arabis mosaic virus clearly has a wide host-range, for raspberry yellow dwarf virus causes severe diseases in raspberry var. Malling Exploit and in several strawberry varieties[3,4]. It also occurs naturally in white clover and in several weed species[3], and it is one of several viruses involved[2] in the etiology of cherry diseases of the rasp-leaf type[5]. Moreover, it has been found on the continent of Europe[2] as well as in widely separated parts of England, Scotland and Wales[3,6].

Little is known about the manner in which soil-borne viruses survive in soil and infect plants, but Hewitt, Raski and Goheen[7] have presented evidence that grape-vine fanleaf virus is transmitted by *Xiphinema index*, a large ectoparasitic, soil-inhabiting nematode with a long mouth-spear. Infections with fanleaf virus occurred when *X. index*, collected from soil around the roots of diseased grape plants, were transferred to pots of sterilized soil in which healthy grape plants were growing. Moreover, the virus persisted with the nematodes when they were added to soil as much as 30 days before the grape plants[8].

Dagger nematodes (*Xiphinema* spp.) were first recorded in Britain in 1959. They were found in Kent[9], in soil collected from the site of an outbreak of arabis mosaic virus in strawberry, and also on land cropped with white clover in Somerset[10]. We too[11] have noted the presence together of *Xiphinema* and arabis mosaic virus at sites in Somerset and Pembrokeshire, and we now present the results of experiments in which the virus was regularly transmitted when dagger nematodes were present but not otherwise. Very recently, Jha and Posnette[12] have also suggested that *Xiphinema* transmits this virus, for some healthy strawberry plants, growing in pots containing pasteurized soil, became infected when suspensions of dagger nematodes, from soil infested with arabis mosaic virus, were added to the pots.

Distribution of *Xiphinema* in Crops affected by Arabis Mosaic Virus

Diseases thought to be caused by arabis mosaic virus were found in widely separated parts of Britain[6]. Outbreaks in raspberry, strawberry and white clover were selected for detailed study of: (*a*) the identity of the viruses involved; (*b*) the distribution of diseased plants; (*c*) the distribution of dagger nematodes; and (*d*) the infectivity of soil from diseased and healthy parts of crops.

The viruses were identified serologically: isolates from each of the sites listed in Table 1 precipitated specifically with an antiserum to arabis mosaic virus. Diseased plants occurred in patches at all the sites. Infected raspberry and strawberry plants showed typical symptoms; infected white clover plants usually had chlorotic blotches on their leaves. The virus was consistently isolated from diseased plants but not from normal-looking plants growing outside the patches. The soil samples were collected during a period of 12 weeks. Aliquots (300 gm.) of top-soil were used either to estimate the numbers of dagger nematodes present, or to grow a crop of pea seedlings (var. Onward) in an insect-proof glasshouse. Four weeks after sowing, the seedlings were washed free from soil and sap from their roots was inoculated to leaves of *Chenopodium amaranticolor* to find how many of the seedlings had become infected: the proportion infected was taken as an estimate of the infectivity of the soil. The dagger nematodes were extracted from the soil by a sieving technique similar to that described by Goodey[13]: large pieces of plant debris were first removed from the muddy soil suspension and this then passed through a 50-mesh sieve, which retained *Xiphinema*. The material

Table 1. Association of *Xiphinema* with Outbreaks of Diseases caused by Arabis Mosaic Virus

Locality	Crop		Soil type	No. *Xiphinema* per 300 gm. soil	Infectivity of soil*
Methwold, Norfolk	Raspberry	(*a*) healthy area	Black peat fen	0	0/2
		(*b*) diseased area	Black peat fen	12	8/8
		(*c*) diseased area	Black peat fen	27	—
Tenby, Pembrokeshire	Strawberry	(*a*) healthy area	Heavy loam	0	0/7
		(*b*) diseased area	Heavy loam	4	5/6
Fareham '*A*', Hampshire	Strawberry	(*a*) healthy area	Light shingle	1	0/3
		(*b*) diseased area	Light shingle	34	4/5
Fareham '*B*', Hampshire	Strawberry	(*a*) healthy area	Light shingle	0	0/8
		(*b*) diseased area	Light shingle	9	7/10
Crewkerne, Somerset	White clover	(*a*) healthy area	Moist sandy loam	0	—
		(*b*) diseased area	Moist sandy loam	35	2/3

* Five pea seedlings were grown for 4 weeks in each 300-gm. aliquot of soil. The incidence of virus infection was then determined by inoculating sap from the pea roots to leaves of *Chenopodium amaranticolor*. Numerator is the number of lots of soil in which pea seedlings became infected, denominator is the total number.

Table 2. Effect of Chemical Treatments on the Nematode Population and Infectivity of Soil

Chemical added to soil*	No. *Xiphinema* per kgm. soil 6 weeks after treatment†		No. living 'small nematodes'‡ per kgm. soil 6 weeks after treatment	Infectivity of soil§ (No. pots containing infected seedlings)
	Living	Dead		
None	21	0	5,029	8/12
Ethylene dibromide (0·4 ml./litre soil)	0	0	1	0/12
Pentachloronitrobenzene (1 gm. 20 per cent dust/litre soil)	0	5	4,608	0/12
Tetramethylthiuramdisulphide (2 gm. 10 per cent dust/litre soil)	0	24	272	0/12

* The mixtures of soils and chemicals were stored in sealed tins for 2 days. They were then potted and pea seeds (5 per pot) planted after a further 2 days. Pentachloronitrobenzene and tetramethylthiuramdisulphide dusts were provided by the Murphy Chemical Co.
† Before treatment the soil contained about 36 *Xiphinema* per kgm. soil.
‡ 'Small nematodes' includes all nematodes that passed through a 50-mesh sieve but were retained on a 160-mesh sieve.
§ Six weeks after planting, the incidence of infection in the pea plants was estimated as described in Table 1. Numerator is the number of pots containing infected seedlings and denominator is the total number of pots used.

extracted on the sieve was examined in a syracuse dish under a binocular microscope. This method was shown to recover at least 75 per cent of the *Xiphinema* in the soil.

Table 1 gives results for a selection of sites, and shows that, although arabis mosaic virus was previously recorded only in soils of medium-heavy texture[4], it is not restricted to these, and that serious disease outbreaks also occur on black fen and shingle soils[6]. All the samples from soil inside patches of diseased plants were infective and all contained *Xiphinema*, whereas soil from outside such patches was not infective and, with one exception, did not contain *Xiphinema*. At the exceptional site, Fareham '*A*', a single dagger nematode was found in 300 gm. soil collected outside the diseased area. Each population of dagger nematodes contained both larvæ and adults, but the proportion of adults varied from less than 5 per cent to 30 per cent at different sites. The total numbers of *Xiphinema* varied considerably between sites but were never large, suggesting that if dagger nematodes are involved in virus transmission, only a few are needed to enable the soil to become highly infective. Indeed, the numbers recovered from five 300-gm. lots of Fareham '*B*' soil, in each of which pea seedlings had become infected, were 3, 4, 5, 9 and 10, respectively: 5, 9 and 17 *Xiphinema* were isolated from three similar lots in which the seedlings had remained healthy. Soils collected from under diseased plants at several additional sites all contained *Xiphinema*.

Tests with Isolated Dagger Nematodes

Results of soil fractionation experiments, in which infective soil was suspended in water and then passed successively through 25, 50 and 160-mesh sieves, showed that each sieve retained infective material but that the fraction of soil passing through the 160-mesh sieve was not infective. The distribution of infectivity roughly paralleled that of the dagger nematodes. Other tests showed that soil extracts of the type used for counting *Xiphinema* (made using a 50-mesh sieve) conferred infectivity on sterilized soil. Attempts were therefore made to separate the dagger nematodes from the other animals and materials in these extracts. The extracts were thoroughly washed on a 160-mesh sieve, re-suspended in water, the dagger nematodes were picked out with a wire handling-needle and transferred, first to watch-glasses containing distilled water, and then to pots containing virus-free soil. The extract residues were added to other similar pots of soil. All the pots were planted with peas, which were tested for the virus four weeks later. No plants growing in virus-free soil or in soil to which the extract residues had been added became infected, but plants growing in soil infested with hand-picked *Xiphinema* did. In three experiments, in which 15–20 dagger nematodes were added to each pot, 2 out of 11, 4 out of 12 and 1 out of 13 pots contained infected pea seedlings. In one experiment the dagger nematodes were separated, in batches of 20, into adult males, adult females and larvæ. Infection occurred with each of two batches of males, with two out of three batches of females but with none of six batches of larvæ. In a second similar experiment, infection occurred with two out of sixteen batches of larvæ but with none of twelve batches of adults. Serological tests on several of the virus isolates from pea seedlings exposed to hand-picked *Xiphinema* confirmed that each was arabis mosaic virus. Isolates from plants exposed either to male or to female *Xiphinema*, or to larvæ, were among those identified serologically.

Chemical Treatment of Infective Soil

The infectivity of soils containing tomato black ring virus is abolished by treatment with each of several different chemicals, some of which seem to have little or no effect on infectivity of the virus *in vitro*[11]. Table 2 shows the result of treating soil containing arabis mosaic virus with some of these substances. After peas had been grown in them for six weeks, no living dagger nematodes were found in any of the soils treated with chemicals, and from the fact that no plant growing in these soils became infected, it seems probable that the *Xiphinema* were either killed quickly or deterred from feeding on the plants. Table 2 also contains two other items of note. First, many dead *Xiphinema* were found at the end of the experiment in soil treated with tetramethylthiuramdisulphide, suggesting either that they had died only recently or that the chemical had prevented their decomposition. Secondly, although counts of other large eelworms, for example, *Dorylaimus* spp., paralleled those for *Xiphinema*, pentachloronitrobenzene (and in some experiments tetramethylthiuramdisulphide) seemed, at the doses used, to have little effect on the numbers of 'small eelworms' (all eelworms that passed through a 50-mesh sieve but were retained on a 160-mesh sieve). In the soil, these chemicals appear to have differential effects on different kinds of nematodes.

Air-drying soil at 20° C. for a week is another treatment that abolishes the infectivity of soil containing arabis mosaic virus[11]. Here again, the effect is most plausibly explained by the treatment killing *Xiphinema*, for after a week of air-drying, only 25 dagger nematodes, all dead, were isolated from 1 kgm. of soil, whereas 64, all alive, were found in a similar lot kept moist.

Conclusions

Throughout our work the infectivity of soils and of preparations from soils has been correlated with the presence of living dagger nematodes. Moreover, the fact that *Xiphinema* was absent or rare outside patches of diseased plants suggests that the nematodes are often the limiting factor in the spread of arabis mosaic virus. We have found *Xiphinema* only in fields where arabis mosaic virus occurs.

In the soils we have used, arabis mosaic virus seems associated only with the dagger nematodes. Neither the residue left after their removal from infective extracts, nor the material passing through a sieve which retained the dagger nematodes, was infective. All the evidence points to *Xiphinema* as being the vector of arabis mosaic virus, and it may seem unnecessarily cautious to mention the possibility that the virus could be associated with the nematodes in such a way that they are not acting as virus vectors in the usual sense. The evidence for virus transmission by the nematodes is as good as, but no better than, that commonly accepted as indicating that insects are vectors of plant viruses. If dagger nematodes are virus vectors in the usual sense, a role for which they seem well fitted, it is clear that they can be quite efficient. For example, the tests made on infective soil from Tenby (Table 1) suggest that about 1 in 3 of the dagger nematodes transmitted the virus. Then again, in one of the experiments with hand-picked dagger nematodes, an estimated 1 in 12 of adults transmitted the virus, despite the probability that the soil and environmental conditions were not optimal for them. Although we found there was no need to provide the nematodes with fresh sources of virus, had this been done, the rates of transmission by hand-picked nematodes might have been higher. This possibility, and the mechanism of virus transmission, are the subject of further investigations.

Losses of crop from arabis mosaic virus can probably be avoided in raspberry by growing immune varieties, such as Malling Jewel[14], but it is uncertain whether other crop species also contain immune varieties. However, if dagger nematodes spread the virus, additional methods of control seem possible. The results of treating soils with chemicals show that *Xiphinema* is as sensitive to them as are some small plant-parasitic nematodes[11], possibly more so, for dagger nematodes were killed by the action of some substances commonly used as fungicides and not as nematicides. The fact that many soils contain only relatively few *Xiphinema* also encourages the view that soil nematicides will be effective in controlling the diseases. The problem is now a practical one, namely, to incorporate the nematicide into soil sufficiently evenly and to a sufficient depth to ensure a high percentage kill of the population at an economically acceptable cost.

The taxonomic status of the dagger nematode used in our experiments is still being studied in detail, but preliminary results (Goodey, J. B., Peacock, F. C., and Pitcher, R. S., unpublished observations) suggest it is closely related to, and possibly identical with, *Xiphinema diversicaudatum* Micoletzky, and thus differs from the one associated with grape vine fanleaf virus in California[7]. Whether the two viruses are serologically related is unknown, but fanleaf virus has not been transmitted to species outside the Vitaceae or by mechanical inoculation of sap. But even if these two viruses are closely related, they are unlikely to be the only ones the spread of which depends on soil-inhabiting nematodes. Indeed, evidence was recently advanced that the transmission of peach yellow bud mosaic virus is associated with *Xiphinema americanum*[15]. Several viruses resemble arabis mosaic virus in possessing a wide host-range, in the type of symptoms they cause, in their *in vitro* properties[14,16] and in having isometric particles of approximately 25 mμ diameter (Harrison, B. D., and Nixon, H. L., unpublished observations). With one of these viruses, tomato black ring, there is already evidence that an agent in soil plays a part in virus transmission[11], and it is among such viruses that additional nematode-transmitted types seem most likely to be found.

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ULTRACENTRIFUGATION AND STAINING OF *HEVEA* LATEX

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COOK AND SEKHAR, in 1953 [1], described the separation of *Hevea* latex into phases by high-speed centrifugation and interpreted their results in terms of the types of particle which had been found by various earlier workers in latex after collection from the tree. In particular, the sedimented material was considered to represent an aggregation of the lutoid particles discovered by Homans and van Gils[2]. Cook and Sekhar's method has been used continually in these laboratories in the preparation of latex fractions for chemical and enzymological investigations[3], and there is no doubt as to the general correctness of their conclusions. However, more recent observations seem to show that the sedimentable material in latex cannot consist wholly of one species of particle, and that the non-sedimentable components of latex are also more complex than was thought hitherto. A short account of these observations seems

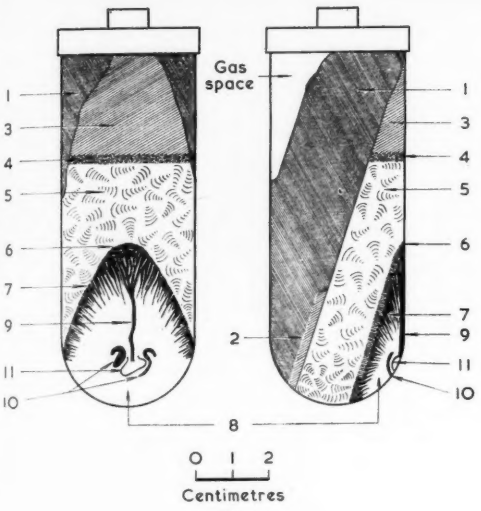

Fig. 1

timely; there is current interest in the biochemistry of *Hevea*[2–6] and high-speed centrifugation of latex is being used in other laboratories besides our own[4].

In the present work, latex was obtained from a small stand of buddings of the clone Prang Besar 186 of *Hevea brasiliensis*. These trees were mature and had been tapped by the half-spiral alternate-daily system[7] for many years. The latex was collected for 30 min. after tapping, into glass vessels kept in ice baths. It was centrifuged at 53,620*g*(max.) for 40 min. in Rotor No. 21 (prechilled to 0°) of a Spinco Model *L* ultracentrifuge. The temperature of the latex thus fell to 0–5° C. soon after leaving the tree and remained in this range virtually throughout the fractionation. Such close temperature control was not used in the earlier work cited[3], although this point is not always clear in the published accounts.

Fig. 1 shows diagrammatically the separated zones regularly observed after centrifugation. Two views of a single tube are shown; these are not cross-sections, but approximate plane projections of the pattern visible from outside the tube. The various zones were distinguishable by colour or texture or both; in a series of experiments extending over ten weeks some variation in detail occurred, but the main features of the pattern were surprisingly reproducible. Further definition of the zones and some indication of their possible cytological significance were obtained by treating the latex with trace amounts of janus green *B* or neutral red before centrifugation. This procedure produced a pattern which seemed to correspond in all essential respects with that formed from unstained latex, and it thus appeared that the stains do not significantly affect the sedimentation behaviour of the particles present.

A full description of the appearance of each of the eleven zones, as observed with and without prestaining the latex, would be rather voluminous. Only the major points will be mentioned here.

Zone 1, which is white and opaque, though shaded in the diagram, corresponds with the 'top white fraction' of Cook and Sekhar[1]. The much smaller zone, 2, is distinguished from it by a certain translucence and, in latex pretreated with janus green *B* by a bright blue colour. (Janus green *B* staining is barely perceptible in zone 1.) Zone 3, a white emulsion, appears cognate with zone 2, since it also stains blue with janus green *B* and on more prolonged centrifugation it is reduced in amount while zone 2 is increased. Zone 4 (orange) is the 'yellow-orange layer' of Cook and Sekhar[1]; the aqueous zone 5 corresponds with the 'C serum' of earlier literature[3] and zones 6–11 are together broadly equivalent to 'bottom fraction'. However, in this connexion it should be noted that the effects of the fully refrigerated technique are considerable: in our experiments zone 5 was turbid, and the sedimented material (6–11) was semi-liquid. If centrifugation is conducted at higher temperatures as in previous work[3], the serum is clearer and the sediment more nearly solid; the stratification of the latter is also less sharp.

Zone 6 is a loose whitish layer on the upper plane of the sediment. Agitation re-disperses it largely into zone 5, where it assumes the same ropy texture as the turbidity initially present. Prolonged ultracentrifugation of zone 5 at about 140,000*g*(max.) deposits a small gelatinous sediment which gives a positive test for pentose (orcinol reaction).

Zone 7, embedded in the face of zone 8, contains both yellow and whitish material and is unstained by neutral red. With janus green *B* the colour of the zone is at first ill-defined, varying from yellowish to dirty green. However, when the tube and contents are allowed to warm to 20–25° after centrifugation, the zone becomes bright red.

Zone 8, which accounts for the bulk of the sediment, is practically a gel at 0–5°, is greyish-cream in colour and becomes syrupy at room temperature. From latex pre-treated with neutral red it is intensely pink and contrasts very strikingly with the unstained zone 7.

Zone 9 is a streak of material resembling zone 7 in appearance and staining reactions, but more strongly sedimented. It joins zone 7 at the upper end of the pellet, and no clear boundary can be drawn. Zone 10 is a ring of deep orange material, and zone 11 a speck of translucent greyish jelly. These last two are minute in amount but their presence is generally quite unmistakable.

All the above results refer specifically to Prang Besar 186 latex, but preliminary experiments on other latexes indicate a similar complexity. Of the particles known to be present in latex after collection, lutoid particles are, by definition[2], readily sedimentable. Also Wiersum[8] has reported that they stain with neutral red (under the microscope). It therefore seems that my zone 8 must contain these particles, a conclusion readily reconcilable with that of Cook and Sekhar. Similarly, zone 1 must still be ascribed to rubber particles and zone 4 to Frey–Wyssling particles[9], as by these authors.

Collected latex also contains bacteria: our samples gave viable counts in the range 10^5 to 5×10^6 organisms per ml., by the method of Taysum[10]. The total volume of bacteria in one centrifuge tube of latex (90 ml.) would probably be small.

To explain the observations outlined above, even partially, it seems necessary to postulate the presence in latex, in addition to the particles already mentioned, of the following: (1) a variety of particle staining blue with janus green *B* and migrating centripetally (zones 2 and 3); (2) at least one species of particle which sedimented with difficulty (zone 6; further centrifugation of zone 5); (3) at least one species of particle reducing janus green *B* (zones 7 and 9).

A more complete explanation would necessarily be speculative; my purpose at this stage is primarily to direct attention to the complexity of collected *Hevea* latex. However, it should ultimately be possible to relate the particulate components of the latex to the normal concepts of plant cytology. With this intention, Wiersum[8] has published evidence suggesting that 'lutoids' may be considered as the elements of a polydisperse vacuome. In my observations the component sedimented with difficulty has suggestive similarities to the particulate ribonucleoprotein ('microsomes') now well known in animal cells and also reported in higher plants[11]. Further, McMullen[3] has recently detected ribonucleoprotein in freeze-dried latex serum. The reaction of zones 7 and 9 with janus green is essentially the same as that reported for liver mitochondria by Recknagel[12] using a closely similar experimental method. Reactions with janus green must, of course, be interpreted with reserve, especially perhaps in the presence of bacteria.

It should also be possible to relate the components of collected latex to the cytology of the lacticifers *in vivo*. The existing literature on this subject, reviewed by Wiersum[8] and Milanez[13], is somewhat controversial, and very little is known of the cytology of latex vessels submitted to regular tapping. Milanez considers that in intact vessels latex is homologous with cytoplasm and not with vacuolar sap, as in the traditional opinion[14], and he has also reported the presence of mitochondria. If these views are applicable to latex collected by tapping, the complexity of latex revealed in the centrifuge would find an explanation.

I am indebted to Mr. W. G. Wren for advice and encouragement, to Ir. P. de Jonge and Mr. D. H. Taysum for valuable discussions and to the last-named also for all bacteriological data and for access to his unpublished microscopic studies on latex. I acknowledge also the technical assistance of Mr. Yap Chin Fah and Mr. Hoh Lian Yong, as well as translations by Dr. J. K. Templeton, Mr. N. G. Bissett and Ir. E. C. Paardekooper. This work, which is continuing, forms part of a programme of research undertaken by the Board of the Rubber Research Institute of Malaya.

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GLYCOPROTEINS OF HUMAN CEREBROSPINAL FLUID

By Dr. SAMUEL BOGOCH

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WHILE the cerebrospinal fluid is the only compartment of the central nervous system which can readily and repeatedly be sampled in both man and animals without unphysiological effects, relatively few quantitative data are available on its organic constituents[1]. Micromethods have been developed in this laboratory which permit the analysis of small quantities of cerebrospinal fluid with special reference to the carbohydrate and protein constituents. A total of 236 individual specimens of cerebrospinal fluid have been examined by these techniques.

10–30 c.c. aliquots of lumbar cerebrospinal fluid are immediately lyophilized, quantitatively dialysed in 'Cellophane' at 4° C. against glass-distilled water to completion, with the result that three major fractions are obtained[2,3]: Fraction *G* (non-dialysable, water-soluble in conditions of vanishing ionic strength); Fraction *P* (non-dialysable, water-insoluble); Fraction II (dialysable). Both the original unfractionated cerebrospinal fluid and the resultant subfractions have been quantitatively analysed for total solids, nitrogen, phosphorus, hexose, hexosamine and reducing sugar. In addition, it has been possible by this method to obtain accurate values for the presence of true neuraminic acid in cerebrospinal fluid, since that which is associated with non-dialysable fractions has been freed from the interference of dialysable monosaccharides, and the neuraminic acid which is unbound and dialysable, when present, can be detected by the use of column chromatography ('Amberlite *IRC*-120' for sodium ions, followed by 'Dowex-3-*OH*' for chloride ions, then 'Dowex-1-formate' for neuraminic acid) to separate it from the large excess of monosaccharides present, and by the thiobarbituric acid reaction[4,5]. The values of total neuraminic acid have been found to vary from 2 to 29 μgm./c.c. of whole cerebrospinal fluid, with from 0 to 12 μgm. of the total representing free neuraminic acid. The previous use of the Bial reagent to measure total neuraminic acid in whole cerebrospinal fluid[6,7] gave a somewhat higher range of values due to monosaccharide interference. Individual differences in the concentration of neuraminic acid in cerebrospinal fluid were detected with the Bial reagent, despite the large interference by monosaccharides, because most of these differences have been shown to be the result of variation in the concentration of Fraction *G* neuraminic acid[2].

Table 1. MOLAR QUANTITIES AND RATIOS OF HEXOSE, HEXOSAMINE AND NEURAMINIC ACID IN FRACTION *G* OF INDIVIDUAL SPECIMENS OF CEREBROSPINAL FLUID

Total solids in Fraction *G* (mgm./c.c.) cerebrospinal fluid	Molar ratios			Molar ratio	
	Hexose (as glucose)	Hexosamine (as galactosamine)	Neuraminic acid	*Hexose : Hexosamine*	
0·338	1·4	5·6	1	0·25	
0·331	1·8	2·8	1	0·65	
0·490	3·5	5·2	1	0·69	
0·530	1·3	1·8	1	0·72	
0·616	2·9	3·8	1	0·76	
0·320	3·8	4·8	1	0·79	
0·658	2·7	3·0	1	0·90	
0·450	3·2	3·6	1	0·91	
0·314	2·2	2·2	1	1·00	(1 : 1)
0·734	2·7	2·7	1	1·00	
0·292	3·7	3·5	1	1·06	
0·323	3·4	3·1	1	1·10	
0·275	4·7	4·1	1	1·15	
0·335	3·8	3·2	1	1·19	
0·159	4·6	3·9	1	1·18	
0·371	3·6	3·0	1	1·20	
0·499	4·2	3·4	1	1·24	
0·150	4·5	3·6	1	1·25	
0·274	5·0	3·8	1	1·32	
0·178	2·9	2·0	1	1·45	
0·278	7·4	4·2	1	1·76	
0·428	6·9	3·9	1	1·76	
0·403	5·4	2·8	1	1·93	(2 : 1)
0·192	5·0	2·3	1	2·17	
0·308	5·7	2·6	1	2·19	
0·424	6·2	2·8	1	2·22	
0·179	3·2	1·4	1	2·28	
0·345	4·3	1·9	1	2·30	
0·354	14·4	6·2	1	2·32	
0·434	6·1	2·6	1	2·34	
0·403	6·6	2·7	1	2·44	
0·374	10·3	3·6	1	2·80	
0·187	5·0	1·7	1	2·94	
0·425	9·9	3·3	1	3·00	(3 : 1)
0·543	7·7	2·4	1	3·20	
0·296	3·3	1·0	1	3·30	
0·278	34·4	9·9	1	3·48	
0·538	5·3	1·3	1	4·07	(4 : 1)
0·142	8·7	2·1	1	4·20	
0·480	6·1	1·1	1	5·55	
0·276	6·7	1·1	1	6·09	(6 : 1)
0·428	13·6	2·2	1	6·18	
0·594	6·7	0·7	1	9·58	(10 : 1)

All the hexosamine in cerebrospinal fluid has been shown to be non-dialysable, most of it being associated with Fraction *G*. The absolute quantities of hexosamine (as galactosamine) range from 1·7 to 60·1 μgm./c.c. whole cerebrospinal fluid (mean 14·4). Fraction *G* hexose (as glucose) has similarly been shown to vary from 4·0 to 88·3 μgm./c.c. whole cerebrospinal fluid (mean 25·7). The distributions of the constituent hexose and neuraminic acid have been studied in electrophoretic subfractions of Fraction *G* and have been shown to be associated mostly with α_2- and α_1-globulin fractions, with some samples showing the greatest concentration of these constituents in the β-globulin fraction.

Optimal conditions have been established for the hydrolytic release of the constituent carbohydrates of Fraction *G*. Utilizing 0·08 *N* hydrochloric acid hydrolysis at 100° C. for 20 min., 65 per cent of the original neuraminic acid of the macromolecular fraction was released in dialysable form, together with 60 per cent of the original hexosamine, but only 8 per cent of the original hexose. Comparison of these results with the hydrolytic behaviour of brain ganglioside[8] suggests that in the cerebrospinal fluid glycoproteins, as in brain ganglioside, the neuraminic acid and hexosamine moieties are more peripherally located or at least more available to mild acidic hydrolysis than are the hexose moieties. Further experiments utilizing 2 *N* hydrochloric acid for different periods of time resulted in the selective liberation of the hexoses, galactose having been shown to be released before mannose. Application of these methods to the structural determination of purified cerebrospinal fluid glycoprotein fractions separated by column chromatography is in progress. The structural relationship of these carbohydrate constituents is of interest with reference to their membrane, receptor and transport functions demonstrated in other studies in this laboratory[9–11].

The quantitative determination of neuraminic acid, hexosamine and hexose has permitted an examination of the molar ratios of these substances in individual cerebrospinal fluid specimens. Rather than finding reasonably uniform molar ratios, wide individual variation has been demonstrated in the ratios hexose : hexosamine : neuraminic acid, ranging from approximately 1 : 1 : 1 to 10 : 1 : 1. Table 1 shows a few examples of the variation observed both in absolute amounts of these constituents and in their relative ratios. That this variation is not a function of the total amount of Fraction *G* present per c.c. of cerebrospinal fluid is also shown in Table 1. The total content of solids in cerebrospinal fluid has been shown to tend to increase with age after the second decade. The concentration of hexosamine derived from Fraction *G* increases during the same period. The concentration of hexose derived from Fraction *G* also increases during this period, but even more greatly than does hexosamine. The specific increment of hexose to hexosamine appears to be more marked in the third and fourth decades, the molar ratios of these constituents tending to approach unity after the fifth decade.

The possible biological significance of unusual total concentrations of macromolecular hexose, hexosamine and neuraminic acid, as well as of unusual molar ratios and electrophoretic distributions of these substances in cerebrospinal fluid in relation to different neuropsychiatric disorders, is considered elsewhere[12]. Perhaps most significant is the fact that there has been demonstrated great individual variation both in the absolute quantities and in the relative ratios of particular carbohydrate constituents of cerebrospinal fluid glycoproteins. Whether this potential for individual variation reflects genetically determined normal characteristics or specific chemical patterns of particular functional or disease processes, or both, is yet to be determined. The possibility exists that an opportunity is indicated by means of similar studies on other compartments of the nervous system for the definition of chemical bases of individuality.

These studies, to be presented in detail elsewhere[13], have been supported in part by grants from the National Institutes of Health (*B*-1221 and *M*-2095) and the Scottish Rite Research Committee, National Association for Mental Health (U.S.).

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FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, December 14

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on 'Why Hi-Fi?', opened by Mr. P. P. Eckersley.

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Mr. P. B. Cornwell: "Insect Control by Gamma Irradiation".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.30 p.m.—Baron G. de Gerlache de Gomery: "The Belgian Antarctic Expedition".

Tuesday, December 15

INSTITUTE OF MARINE ENGINEERS (at the Memorial Building, 76 Mark Lane, London, E.C.3), at 5.30 p.m.—Mr. K. Maddocks: "Some Aspects of Marine Reactor Security".

INSTITUTE OF PHYSICS (at 47 Belgrave Square, London, S.W.1), at 5.30 p.m.—Mr. K. G. Freeman: "Tubes for Colour Television".

INSTITUTION OF ELECTRICAL ENGINEERS, MEASUREMENT AND CONTROL SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Data Handling Problems in Atomic Installations", opened by Dr. D. Taylor.

Wednesday, December 16

POWDER METALLURGY JOINT GROUP OF THE IRON AND STEEL INSTITUTE AND THE INSTITUTE OF METALS (in the Hoare Memorial Hall, Church House, Great Smith Street, London, S.W.1), at 9.30 a.m.—Symposium on "The Powder Metallurgy of Magnetic Materials".

ROYAL METEOROLOGICAL SOCIETY (at 49 Cromwell Road, London, S.W.7), at 5 p.m.—Dr. H. Wexler: "Seasonal and other Temperature Changes in the Antarctic Atmosphere"; Mr. W. Hitschfeld: "Patterns of Hailstorms in Alberta".

ROYAL STATISTICAL SOCIETY (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.15 p.m.—Sir Hugh Beaver, K.B.E.: "Statistics as a Tool of Management" (Presidential Address).

INSTITUTE OF PETROLEUM (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. A. C. Hartley: "Pipelines".

INSTITUTION OF MECHANICAL ENGINEERS (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Dr. S. G. Hooker: "The Future of Air Breathing Engines in Aviation" (James Clayton Lecture).

SOCIETY OF CHEMICAL INDUSTRY, CORROSION GROUP (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Discussion of Research Papers recently published by Members of the Group.

SOCIETY OF INSTRUMENT TECHNOLOGY (at Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Mr. J. F. Coales: "Random Inputs and Load Variations".

Thursday, December 17

BRITISH COMPUTER SOCIETY, LONDON BRANCH (at the Northampton College of Advanced Technology, St. John Street, London, E.C.1), at 2.30 p.m.—Discussion on "Conversion between Analogue and Digital Representation".

SOCIETY OF CHEMICAL INDUSTRY, ROAD AND BUILDING MATERIALS GROUP (at 14 Belgrave Square, London, S.W.1), at 2.30 p.m. and 5.30 p.m.—Scientific Papers.

INSTITUTION OF MINING AND METALLURGY (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. A. J. Robinson: "Relationship Between Particle Size and Collector Concentration"; Mr. D. Borchers: "Deepening of No. 1 Vertical Shaft at Premier (Transvaal) Diamond Mining Co., Ltd.".

LONDON MATHEMATICAL SOCIETY (at the Royal Astronomical Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Prof. E. V. Schenkman: "Supersolvable Groups".

INSTITUTION OF ELECTRICAL ENGINEERS, EDUCATION DISCUSSION CIRCLE (at Savoy Place, London, W.C.2), at 6 p.m.—Discussion on "The Presentation of the History and Philosophy of Science", opened by Prof. H. Dingle.

Thursday, December 17—Friday, December 18

PHYSICAL SOCIETY (in the Townsend Lecture Theatre, The Clarendon Laboratory, Oxford), at 10 a.m. daily—Conference on "Electron–Phonon Interaction in Solids".

Friday, December 18

SOCIETY OF CHEMICAL INDUSTRY, FINE CHEMICALS GROUP (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. F. E. Camps: "Some Aspects of Forensic Science".

Friday, December 18—Saturday, December 19

BRITISH PSYCHOLOGICAL SOCIETY (at University College, Gower Street, London, W.C.1)—Conference.

Saturday, December 19

BIOCHEMICAL SOCIETY (in the Department of Chemical Pathology, King's College Hospital Medical School, Denmark Hill, London, S.E.5), at 10.30 a.m.—389th Meeting.

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURER (with a good honours degree and teaching experience) IN CHEMISTRY at the Nigerian College of Arts, Science and Technology—The Council for Overseas Colleges, 12 Lincoln's Inn Fields, London, W.C.2 (December 21).

TECHNICIAN OR SENIOR TECHNICIAN IN THE DEPARTMENT OF PATHOLOGY, University of Khartoum—The Registrar, University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (December 22).

LECTURER IN MATHEMATICS in St. Salvator's College—Joint Clerk to the University Court, College Gate, St. Andrews (December 31).

LECTURER IN STATISTICS in St. Salvator's College—Joint Clerk to the University Court, College Gate, St. Andrews (December 31).

LECTURER (with a special knowledge of, and interest in, the vertebrates) IN ZOOLOGY—The Registrar, King's College (University of Durham), Newcastle-upon-Tyne 1 (December 31).

SCIENTIFIC OFFICER (with a first- or second-class honours degree in civil engineering or physics, or equivalent qualifications) at the Hydraulics Research Station, D.S.I.R., Howberry Park, Wallingford, Berks, for studies of hydraulic problems in the field of civil engineering, including scale model investigations—The Ministry of Labour and National Service, Technical and Scientific Register (K), 26 King Street, London, S.W.1, quoting E.489/9A (December 31).

TECHNICIAN (preferably with experience in a laboratory concerned with the physiology, biochemistry or the culture of micro-organisms) IN THE BOTANY DEPARTMENT, University of Canterbury, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (December 31).

SENIOR LECTURER IN CLINICAL BIOCHEMISTRY at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (January 4).

SENIOR LECTURER (with main interest and special qualifications in the field of petrology) IN GEOLOGY at the University of Western Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (January 4).

READER IN PHYSIOLOGY at Middlesex Hospital Medical School—The Academic Registrar, University of London, Senate House, London, W.C.1 (January 20).

ASSISTANT LECTURERS (with at least a good honours degree in physics, and preferably experience in teaching and/or research) IN PHYSICS at the University of Malaya, Kuala Lumpur—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1.

BIOCHEMIST, Basic grade (with a first- or second-class honours degree in chemistry or biochemistry) IN THE UNIVERSITY DEPARTMENT OF MEDICINE, Glasgow, for research work—The Secretary, Royal Infirmary, 84 Castle Street, Glasgow, C.4.

ISOTOPE TECHNICIAN (preferably with a knowledge of biochemistry or electronics) for work in a specialized laboratory dealing with diagnosis, treatment and research in thyroid disorders—The Secretary, New End Hospital, Hampstead, London, N.W.3.

LECTURER IN VETERINARY ANATOMY at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, February 27).

MASTER to teach Mathematics up to advanced level, with possible scholarship work—The Headmaster, Tonbridge School, Tonbridge, Kent.

SPECIALIST SUPERINTENDENT (CHEMIST) (with a university degree in chemistry or agricultural chemistry, the higher national certificate in chemistry, or the diploma of the Science Technologists Association, or equivalent qualifications) IN THE DEPARTMENT OF AGRICULTURE, Northern Region of Nigeria, to be responsible for the organization, supervision, and general running of the Chemistry Laboratories at Samaru, and also to undertake certain determinations for research projects—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD/63/408/018/T.

TECHNICIAN (female, with manual dexterity for preparing small fossils) IN THE ZOOLOGY DEPARTMENT—The Secretary, University College London, Gower Street, London, W.C.1, quoting Ref. No. Zoo/4.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Some Aspects of Agricultural Research: a Reprint of the articles in the Report of the Agricultural Research Council for the year 1957-1958. Pp. iii+67. (London: H.M. Stationery Office, 1959.) 3s. 6d. net. [2210

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LETTERS TO THE EDITORS

ASTRONOMY

Absence of Craters on the Far Side of the Moon

THE recent photographs of the far side of the Moon taken by the Russian lunar rocket *Lunik III* raise interesting possibilities regarding the origin of lunar craters and the interplanetary material within our solar system the impact of which on the Moon's surface is presumed to cause the craters. If the reported lower incidence of craters on the Moon's far side is real and not a result of inadequate photographic resolution, it may be readily explained by the effect of the Earth's gravitation field on meteoric material orbiting about the Sun.

Material passing near the Earth will be deflected through an angle θ given by :

$$\theta = 2\cot^{-1}(2ES/GmM) \sim R/KS \qquad (1)$$

where E is the particle's kinetic energy with respect to the Earth ($=[1/2]\mathrm{mV}.^2$), S is the perpendicular distance from the Earth's centre to the original trajectory of the particle, G is the universal gravitational constant, M and R are the mass and radius of the Earth, and K is a convenient measure of the particle energy in terms of the particle's potential energy with respect to the Earth at one Earth radius. If the velocity of the material with respect to the Earth-Moon system were isotropic, no observable effect would result since material scattered by the Earth on to the Moon's surface would be compensated by an equal quantity of material originally aimed at the Moon scattered away from the Moon by the Earth. The velocity of the material with respect to the Earth-Moon system is isotropic only in the plane of the Moon's orbit about the Earth due to the motion of the Moon in its orbit. Since the particles orbit about the Sun, their directions relative to the plane of the ecliptic (plane of the Earth's orbit) are uniquely related to their perpendicular distance to the ecliptic plane at the Earth's orbit.

Therefore, scattering components in the ecliptic plane, where the particle velocities are isotropic, have no effect on particle flux on the Moon's surface. Scattering components perpendicular to the ecliptic plane, however, double the incidence of particles hitting the Moon by directing particles above and below the Earth towards the Moon where only particles in the ecliptic plane would have hit the Moon in the absence of the Earth's attractive field. Particles near the ecliptic plane but incident at large angles with the Earth-Moon line will not be appreciably affected if their energy is such that their scattering towards the ecliptic plane does not carry them a lunar radius nearer the Moon than they ordinarily would have gone. Stated briefly in terms of the particle energy, K, the ratio of the number of craters on the Earth side of the Moon to the number on the far side is given by 2 or $(1+2{\cdot}6/K)$ whichever quantity is less. Material within the solar system at the present time moves relative to the Earth with K's of the order of unity as observed and interpreted in scattered sunlight[1] and meteor radiants[2]. If there is an appreciable scarcity of craters on the Moon's far side, one may conclude that the motion of interplanetary material has not changed appreciably during the time since the formation of the Moon's surface and that lunar craters were not formed by material from outside our solar system.

DAVID B. BEARD

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Nov. 3.

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CHEMISTRY

Sensitivity of Catharometers in Gas Chromatography and the Thermal Conductivity of Binary Gas Mixtures

IN a recent communication, Ray[1] has given the equations :

$$\frac{dT}{dx_2} = \frac{\text{const.}}{\lambda_1^2}\cdot\frac{d\lambda_{12}}{dx_2} \quad (1) \text{ and } \frac{d\lambda_{12}}{dx_2} \simeq \lambda_1 - \lambda_2, \quad (2)$$

which he then combines and discusses. In these equations, the left-hand side of (1) is proportional to the sensitivity of a catharometer ; λ_1 and λ_2 are the thermal conductivities of carrier gas and vapour respectively ; λ_{12} is the thermal conductivity of the mixture of the carrier gas with a fraction (assumed here to be molar) x_2 of vapour. Authors discussing this communication[2] all appear to assume that equation 2 is sufficiently accurate to enable them to use it in discussion of experimental results. This assumption is not correct.

Equations for the thermal conductivity of binary gas mixtures based on the Chapman-Enskog theory of transport properties are given, *inter alia*, by Chapman and Cowling[3], who also give an equation (13·5.2, ref. 3) for the case that the proportion of one component (x_2) is small. This equation, which replaces equation 2, is of the form :

$$\frac{d\lambda_{12}}{dx_2} = \lambda_1\left[\frac{-Q\lambda_1/F + P + F/\lambda_1}{R}\right] \quad (x_2 \text{ small}) \quad (3)$$

where P, Q, R and F are calculable functions of molecular properties. This equation does not include λ_2 ; it is in fact unreasonable that λ_2, the value of which is determined by collisions between molecules of pure vapour, should appear in an equation for λ_{12} in a gas where such collisions are virtually absent.

If helium is used as a carrier gas for organic vapours the molecular weight of which is greater than about 70, equation 3 can be much simplified, for in this case P, Q and F are such that the first term is greater than at least 50 times the sum of the second and third, so that the latter may be neglected. If it is assumed that both carrier and vapour molecules can be represented by hard spheres of diameter σ_1 and σ_2 respectively, and also that the transport

properties are not affected by the internal energy of the vapour molecules, then substitution of equations 9·82.1, 10·21.1, 10·22.1, and 10·22.2 into 13·5.2, into 13·5.2, and omission of relatively small quantities gives :

$$\frac{-1}{\lambda_1}\frac{d\lambda_{12}}{dx_2} \simeq 2{\cdot}3\,\frac{\sigma_{12}^2}{\sigma_1^2}\left[\sigma_{12}=\frac{\sigma_1+\sigma_2}{2}\right] \qquad (4)$$

A proportionality equivalent to equation 4 has been given by Hoffmann[4], working from classical kinetic theory. This equation illustrates how the vapour acts in changing the thermal conductivity of the carrier. The vapour molecules are large, heavy and slow, whereas those of the carrier are small, light and fast. Virtually all the heat is carried by the carrier molecules, and the vapour molecules act merely by getting in the way, which they do in proportion to their total cross-sectional area, given by $x_2\sigma_{12}^2$.

From equations 1 and 4, the sensitivity of the catharometer for vapours in helium is proportional to σ_{12}^2. Values of this for a few molecules are available in the literature, but there are not enough to be of general use, and furthermore, the concept of molecular diameter is not sufficiently precise to make such values applicable in all circumstances. By analogy with the case of ionization in a binary gas mixture, however, it seems possible that the cross-sectional areas of organic molecules are approximately equal to the sum of the cross-sectional areas of their component structural units. Study of the sensitivity of a catharometer published by Rosie and Grob[5] supports this possibility. Table 1 gives sensitivities relative to benzene found by Rosie and Grob. The

Table 1

Vapour	Relative molar sensitivity: Rosie and Grob	Relative molar sensitivity: Calculated	Vapour	Relative molar sensitivity: Rosie and Grob	Relative molar sensitivity: Calculated
n-Pentane	105*	—	Ethylbenzene	129	135
n-Heptane	143	143	*o*-Xylene	130	132
n-Octane	160	162	*p*-Xylene	131	132
n-Decane	199*	—	*iso*-Propylbenzene	142	150
2:2-Dimethylbutane	116*	—	Mesitylene	149	148
2:3-Dimethylbutane	116*	—	*cyclo*-Pentane	97	95
2-Methylpentane	120	120	Methyl*cyclo*pentane	115	110
3-Methylpentane	119	120	1:1-Dimethyl*cyclo*-pentane	124	120
2:2-Dimethylpentane	133	135	*cyclo*Hexane	114	114
2:3-Dimethylpentane	135	135	1:2:4-Trimethyl-*cyclo*pentane (*Cis-trans-cis*)	136	140
2-Methylhexane	136	139	1:2:4-Trimethyl-*cyclo*pentane (*Cis-cis-trans*)	143	140
3-Methylhexane	133	139			
3-Ethylpentane	131	139			
2:2:4-Trimethyl-pentane	147	150			
Benzene	100*	—			
Toluene	116*	—			

Experimental results in Table 1 copied by courtesy of *Analytical Chemistry*.

starred entries of this Table have been used to work out the cross-sectional areas (relative to benzene) of the structural groups listed in Table 2, which gives the values found. The rest of Table 1 then compares experimental sensitivities with those calculated from

Table 2. Group Cross-Sections

$-CH_3$	24	$-\overset{|}{\underset{|}{C}}-$	1
$>CH_2$	19	$-C_6H_5$	92
$>CH$	10	$-H$ (aromatic)	8

Table 2. Both sets of figures agree well, except in cases such as 3-ethylpentane, where the molecule is unusually compact. In such cases, and in the case of the last two isomers in the Table, terms depending on the structure should be added, but there are insufficient results to be able to do this. This procedure clearly provides a method for the semi-empirical calculation of the sensitivity of a catharometer for quantitative gas-chromatographic analysis.

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The Iron-Binding Centre of Ferrichrome Compounds

Isolation during *Ustilago sphaerogena* fermentation of the crystalline ferric co-ordination compounds ferrichrome and ferrichrome *A* has been reported previously[1,2]. The formation of these and similar products as a function of the iron nutrition of micro-organisms has been described[3] and the available information relative to their structure and function has been reviewed[4]. The only member of this series which has been isolated, crystallized and characterized by synthesis is the substance produced by *Bacillus subtilis*, that is, the glycine conjugate of 2,3 dihydroxybenzoic acid[5].

In the present communication we wish to report evidence which indicates that the ferrichrome compounds are ferric hydroxamates.

(1) *Hydroxylamine content.* Samples of ferrichrome and ferrichrome *A* were hydrolyzed in dilute sulphuric acid in sealed tubes at 106° for 12 hr. The liberated hydroxylamine was determined by the Csaky method[6]. Authentic acethydroxamic acid was used as a control since slight destruction of the hydroxylamine occurred during the course of hydrolysis. In duplicate analyses the corrected values found were 2·86 and 2·92 moles for ferrichrome and 3·08 and 2·94 moles for ferrichrome *A* per gram atom of iron. The hydrolysates were subjected to paper chromatography with ethanol or methanol and dilute hydrochloric acid as solvent systems. The presence of free hydroxylamine was confirmed by application of the ammoniacal acetylmonoxime nickel spray of Bremner[7] and the tetrazolium spray of Snow[8]. Bound hydroxylamine was also found in grisein and Terregen's factor but not in hydrolysates of ferritin and conalbumin.

(2) *Spectral data.* The broad absorption bands of ferrichrome and ferrichrome *A* with maxima at 425 and 440 mμ and ε_M of 2895 and 3740, respectively, compare favourably with those for ferric acethydroxamate with a maximum at 420 mμ and ε_M of 2890 and ferric benzohydroxamate with a maximum at 435 mμ and ε_M of 4050. These data are for *p*H 7, and, in the case of the model ferric hydroxamates, a large excess of ligand was used. In very dilute acid the band for the model ferric hydroxamates was diminished in intensity and shifted almost 100 mμ to the red. A somewhat similar behaviour was found for the ferrichromes except that much stronger acid was required in order to alter the spectrum. In the *p*H range 7–11 the ultra-violet end absorption of

acethydroxamic acid was strengthened and moved to longer wave-lengths, the rate of change being greatest in the region of the *pKa* (9·4). Comparable results were found for iron-free ferrichrome but with iron-free ferrichrome *A* the intense background absorption obscured the results.

(3) *Ligand properties.* Iron-free ferrichrome and ferrichrome *A* are very weak acids which consume three equivalents of strong base with *pKa'* of about 9. The latter value falls within the range reported for simple hydroxamic acids[9]. In the presence of ferric ion this titration zone is completely eliminated and the re-synthesized complexes, like the natural products, are not decomposed by dilute acid or ammonium hydroxide. Ethylenediaminetetraacetic acid is able to bleach only slowly and incompletely the yellow colour of the ferrichromes; ferric acethydroxamate, on the other hand, is instantly and completely decolourized by similar treatment. No colour is produced with ferrous iron, and a complex, if it exists, must be relatively weak. The cupric complexes of the iron-free ferrichromes exhibit the characteristic extreme insolubility of the copper hydroxamates. The ferric hydroxamate structure for ferrichrome and ferrichrome *A* is compatible with Ehrenberg's[10] magnetochemical measurements in which he identified both products as spin-five 'ionic-type' ferric complexes.

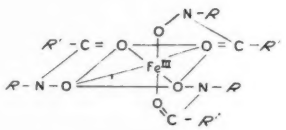

Fig. 1. The iron-binding centre of the ferrichrome compounds

From the above data it is evident that the iron-binding centre of the ferrichrome compounds can be formulated as a trihydroxamic acid (Fig. 1). Some results[4] indicate that these substances are polydentate ligands, the three hydroxamic functions and one iron atom being attached to one molecule. This arrangement of atoms probably results in a considerable chelate effect and this in turn serves to protect the complexes against acid and ethylenediaminetetraacetic acid. In the structure shown, which accounts for about 30 per cent of the molecule, *R* may be either a hydrogen atom or some easily detached group. The three hydroxamic acid functions are inter-connected through *R* or *R'*, the nature of which are under investigation.

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High-Voltage Paper Electrophoresis of Aliphatic Amines and Related Compounds

Successful separations of amines by paper electrophoresis were first described by R. Weber[1] who applied a potential gradient of 10 V./cm. at *p*H 3·8. Several authors[2] have since reported on the usefulness of the technique for resolving mixtures of simple, biogenic and poly-amines. All, except J. Gayer[3], have used low to moderate potential gradients and varying electrolyte solutions.

The multitude of amines potentially present makes their separation and reliable identification in unknown mixtures quite difficult. The application of high potential gradients with attendant increased resolving power and high degree of reproducibility offers obvious advantages. The design of suitable equipment[4] allowing the simultaneous running of up to 12 samples, even when applied as streaks, has facilitated sharp separations of closely neighbouring members of a homologous series and their more certain identification by means of sufficient reference compounds. Although an applied voltage of 5 kV. (100 V./cm.) was found ample, requiring a running time of only 15–20 min. even for a complex mixture, voltages of up to 8 kV. (160 V./cm.) have frequently been used, with about the same separating efficiency, but a shorter running time of 10–15 min. The absence of diffusion and fluid flow effects and the rigorously controlled temperature and pressure conditions can be considered mainly responsible for the satisfactory results.

Some of the attainable separations of lower members of certain homologous series are shown in Fig. 1. Their migration-rates and relative positions can be appreciated from the position of the ammonium ion.

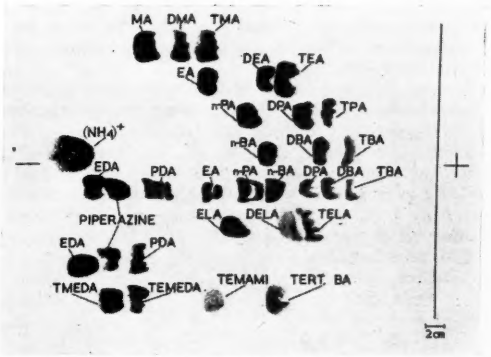

Fig. 1. Electrophoresis of aliphatic amines. Conditions: Whatman No. 3 *MM*, 12×22½ in., 100 V./cm., 5 m.amp./cm., 0·75 *M* formic acid solution, *p*H 2·0, cooling water temperature 8°C., pressure 1·5 lb./in.², 20 min., 0·5 per cent (w/v) ninhydrin in acetone, 5–20 μgm. of each compound.
Amines: *n–BA*, *n*-butylamine; *TERT.BA*, tert.butylamine; *DBA*, dibutylamine; *DEA*, diethylamine; *DELA*, diethanolamine; *DMA*, dimethylamine; *DPA*, dipropylamine; *EA*, ethylamine; *EDA*, ethylene-diamine; *ELA*, ethanolamine; *MA*, methylamine; *n-PA*, n-propylamine; *PDA*, propylene-diamine; *TBA*, tributylamine; *TEA*, triethylamine; *TELA*, triethanolamine; *TEMAMI*, tetramethylammonium iodide; *TEMEDA*, tetramethylene-diamine; *TMA*, trimethylamine; *TMEDA* or *TMDA*, trimethylene-diamine; *TPA*, tripropylamine

The sharpness of separations of 2 homologous series of amines and piperazines is demonstrated in Fig. 2. Although J. T. Edwards[2] had indicated in graph form the feasibility of separating the members of the monoalkylammonium series, an actual separation of a mixture of alkylamines from C_1–C_{10} has never been demonstrated. The slight retardation in

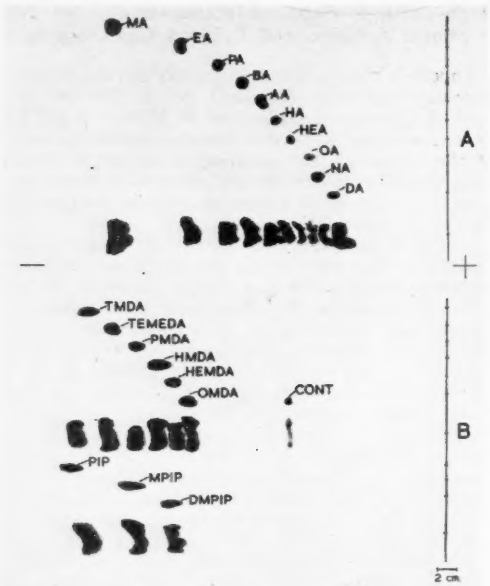

Fig. 2. Electrophoresis of some homologous series of amines and piperazines. Conditions: Same as in Fig. 1, except for cooling water temperature 12°C., and running time 17 minutes.
Mono-amines: *AA*, amylamine; *DA*, decylamine; *HA*, hexylamine; *HEA*, heptylamine; *NA*, nonylamine; *OA*, octylamine;
Diamines: *CONT*, contaminant; *HEMDA*, heptamethylene-diamine; *HMDA*, hexamethylene-diamine; *OMDA*, octamethylene-diamine; *PMDA*, pentamethylene-diamine.
Piperazines: *DMPIP*, trans—2,5, dimethylpiperazine; *MPIP*, 2-methylpiperazine; *PIP*, piperazine

the migration-rates of the compounds in the mixture, compared with the individual migration rates, is a concentration effect due to heavier loading and mutual ionic interference.

It may be added that the high-voltage technique has been recently also successfully applied to mixtures of polyamines, glyoxalines and pyrroles.

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BIOCHEMISTRY

Synthesis of a Protected Heptapeptide Related to the Histidine Analogue of Hypertensin II

Hypertensin II or isoleucine hypertensin II, a pressor peptide with the amino-acid sequence Asp.Arg.Val.Tyr.Is.His.Pro.Phe. has been synthesized along classical lines by two groups of investigators[1].

This octapeptide hormone, in contrast to oxytocin in which the intact 20-membered disulphide ring structure is basic for activity[2], is a straight chain polypeptide. Due to its simple structure and important biological function, hypertensin is very suitable for studying the relationship between chemical structure and biological activity. Moreover, the accumulated evidence for the existence of a peptide-like material with hypotensive activity[3] stimulates new interest for the synthesis of hypertensin analogues.

This communication reports the synthesis of the protected heptapeptide carbobenzoxy-im.benzyl-L-histidyl-L-valyl-L-tyrosyl-L-isoleusyl-im.benzyl-L-histidyl-L-prolyl-L-phenylalanine benzyl ester, related to the histidine analogue of hypertensin II.

The synthesis of the histidine hypertensin analogue, in which the arginine residue is replaced by histidine, has been of especial interest since the finding that the basicity of the amino-acid residue in the side chain of vasopressin[4] has a strong influence on the degree of pressor activity. Consequently, it was considered of interest to investigate whether replacement of the strongly basic arginine residue of hypertensin II by a weakly basic amino-acid (histidine) influenced the pressor activity of the hormone.

Asp.Arg.Val.Tyr.Is.His.Pro.Phe.
Hypertensin II
Asp.His.Val.Tyr.Is.His.Pro.Phe.
Histidine hypertensin II

Recently developed methods were used for the preparation of the protected heptapeptide. Thus, trityl-L-valine[7] was coupled with L-tyrosine methyl ester, by the dicyclohexyl-carbodiimide method and the oily product was detritylated[5] to give L-valyl-L-tyrosine methyl ester hydrocloride (I), in 40 per cent yield, $[\alpha]_D^{25}+32°$ (*c.*, 1 in methanol), R_F 0·91 in *n*-butanol-acetic acid-pyridine-water system[8]. Calculated for $C_{15}H_{23}N_2O_4Cl$: N 10·82, Cl 13·79; found: N 10·50, Cl 13·51.

Carbobenzoxy-im.benzyl-L-histidine[6] was coupled with I in dimethylformamide using dicyclohexyl-carbodiimide as the condensing agent[9]. The product, carbobenzoxy-im.benzyl-L-histidyl-L-valyl-L-tyrosine methyl ester (II) was precipitated with water, yield 73 per cent, m.p. 192–194°C. (all melting points are uncorrected). Calculated for $C_{36}H_{41}N_5O_7$: C 65·93, H 6·30, N 10·68; found: C 65·82, H 6·25, N 10·63.

Hydrolysis of II with *N*-sodium hydroxide followed by acidification with acetic acid produced the acid, carbobenzoxy-im.benzyl-L-histidyl-L-valyl-L-tyrosine (III), in 80 per cent yield, m.p. 185–186°C. Calculated for $C_{35}H_{39}N_5O_4$: C 65·50, H 6·12, N 10·90; found: C 65·45, H 6·10, N 10·99.

Carbobenzoxy-L-isoleucine[10] was coupled by the mixed anhydride method with im.benzyl-L-histidine benzyl ester *p*-toluenesulphonate[9] and the product, carbobenzoxy-L-isoleucyl-im.benzyl-L-histidine benzyl ester[11] was saponified with *N*-sodium hydroxide. Acidification with acetic acid afforded the acid, carbobenzoxy-L-isoleucyl-im.benzyl-L-histidine (IV), $[\alpha]_D^{26}+23·5°$ (*c.*, 1·1 in glacial acetic acid), m.p. 172–174°C. (softens at 138–140°C.). Calculated for $C_{27}H_{32}N_4O_5$: C 65·83, H 6·54, N 11·33; found: C 65·40, H 6·32, N 11·02.

Carbobenzoxy-L-proline was coupled by the mixed anhydride method with L-phenylalanine methyl ester and the oily product was hydrogenated in the presence of palladium oxide, or palladium chloride in methanol containing one equivalent of hydrogen chloride. The product, L-prolyl-L-phenylalanine methyl ester hydrochloride (V) was obtained in 73 per cent yield, m.p. 158–159°C. (reported[1] 157–158°C., 162–164°C.).

Compound IV was suspended in methylene chloride and dissolved by addition of one equivalent of triethylamine. To this solution the equivalent amount of V dissolved in methylene chloride was

added, followed by addition of carbodiimide. After 12 hr. the mixture was filtered and the solvent evaporated. The residue was taken up in ethyl acetate and treated as previously described[11]. The syrupy tetrapeptide ester was saponified to give the acid (amorphous), which was further hydrogenated in the presence of palladium oxide in ethanol. When the evolution of carbon dioxide ceased, the hydrogenation was discontinued, and water was added; on heating all the precipitate dissolved. The catalyst was filtered and the solution evaporated *in vacuo*. The residue was dissolved in acetone and upon addition of ether the product L-isoleucyl-im.benzyl-L-histidyl-L-prolyl-L-phenylalanine (VI) precipitated, $[\alpha]_D^{26} + 19°$ (*c.*, 1 in glacial acetic acid), R_F 0·76 in *sec.* butanol-formic acid-water[12] 0·76; R_F 0·78 in *n*-butanol-acetic acid-pyridine-water. Calculated for $C_{33}H_{42}N_6O_5 \cdot 2H_2O$: C 62·0, H 7·26, N 13·15; found: C 62·0, H 7·27, N 13·20.

Compound VI upon treatment with benzyl alcohol in the presence of *p*-toluenesulphonic acid was converted to its benzyl ester di-*p*-toluene-sulphonate almost in quantitative yield, R_F 0·95 in *n*-butanol-acetic acid-pyridine-water. Calculated for $C_{54}H_{64}N_6O_{11}S_2 \cdot H_2O$: N 8·1; found: N 7·85. This esterification process has been also successfully applied in the synthesis of lysyl peptide benzyl esters during our recent studies[13] dealing with the inhibitory role of phosphoserine adjacent to a lysine residue.

The above tetrapeptide benzyl ester (1·48 gm.) was condensed with tripeptide III (0·8 gm.) by the carbodiimide method in dimethylformamide. The solvent was then evaporated, the residue taken up in ethyl acetate, washed, and the ethyl acetate distilled off. The remaining product, carbobenzoxy-im.benzyl-L-histidyl-L-valyl-L-tyrosyl-L-isoleucyl-im.benzyl-L-histidyl-L-prolyl-L-phenylalanine benzyl ester was filtered by addition of ether. Yield 0·98 gm. (60 per cent crude product). Recrystallized twice from ethanol-ether melted at 145–147°C., $[\alpha]_D^{25} -27°$ (*c.*, 1 in glacial acetic acid). Calculated for $C_{75}H_{85}N_{11}O_{11}$: C 68·47, H 6·50, N 11·71; found: C 68·20, H 6·35, N 11·56. Paper chromatography of the hydrolysate revealed the expected amino-acids in about equal amounts, with the exception of im.benzyl-L-histidine, which gave a more intense spot.

Further details, and the final attempt towards the total synthesis of the histidine analogue of hypertensin II, will appear in a subsequent communication elsewhere.

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Chromatographic Identification of the Antilipæmic *X* Factor of the Unsaponifiable Fraction of Crude Vegetable Oils

SEVERAL workers[1,2] hold that there is some unknown factor in crude corn oil and other vegetable oils which are more effective in lowering the elevated serum cholesterol and phospholipid levels in atherosclerosis rather than purified essential fatty acids alone. Jensen *et al.*[3] reported that crude corn oil contains an unidentified nutritional factor which is more beneficial in increasing the egg weight and hatchability in the hen than essential fatty acids. Recently, Grande *et al.*[4] have shown that there is a factor *X* present in the unsaponifiable fraction of crude corn oil, which can be taken as a better antilipæmic agent than purified essential fatty acids.

It has recently been reported from this laboratory that glucose *cyclo*acetoacetate, a product of condensation of glucose with ethyl acetoacetate, which has been shown by Gonzales and Aparacio[5] to be 2-tetrahydroxy butyl, 5-methyl, 4-carbethoxy furan, on hydrolysis with 2*N* hydrochloric acid gives rise to 1 : 2 dienol glucose[6]. Hydrolysed glucose *cyclo*acetoacetate has also been shown to have a pronounced effect in maintaining the normal levels of cholesterol, and phospholid, and also the C/P ratio in serum and tissues in different kinds of experimental atherosclerosis (ref. 7–9). It is more effective than the essential fatty acids, vitamin B_{12} or inositol. It has also recently been observed that this substance gives a blue colour with molybdenum reagent in acid medium[6] and this test is positive with the unsponifiable fraction of crude linseed oil[9]. Further, in combination with essential fatty acids, this compound has been found to be similar to crude linseed oil in that it lowers the C/P ratio in atherosclerosis induced by saturated fats in rabbits better than the essential fatty acids alone.

In view of the fact that 1 : 2 dienol glucose shows a greater beneficial effect than that caused by essential fatty acids or vitamins B_{12} and because that effect is similar, in some way or other, to that of crude linseed oil, groundnut oil or sesame oil in different kinds of hypercholesterolæmia and experimental atherosclerosis induced by saturated fats[7,9], it has been thought desirable to study whether the unknown *X* factor of the crude vegetable oil bears any resemblance with the 1 : 2 dienol glucose when subjected to circular paper chromatography.

The unsaponifiable fractions of corn oil, groundnut oil, linseed oil and dalda (hydrogenated groundnut oil) were extracted by the method of Grande *et al.*[4] The fractions were purified by removing the colouring matters and other impurities with 'Norite' followed by fuller's earth. 20 μl. of the purified unsaponifiable fraction was spotted on Whatmann No. 1 filter paper of 11 in. diameter with an inner circle of 1 in. radius. The technique of Nath and Bhattathiry[6] using butanol/acetic acid/water (4 : 1 : 5) was followed and the colour developed with phosphomolybdic acid reagent.

Fig. 1 shows that 1 : 2 dienol glucose has the same R_F value (0·92) as that of the *X* factor of the unsaponifiable fractions of corn oil and groundnut oil. Dalda (hydrogenated groundnut oil) did not, however, show the presence of this band, thus indicating that the potent *X* is destroyed by hydrogenation. It is interesting to note that glucose *cyclo*acetoacetate on acid hydrolysis for more than 15 min. gives rise to another band lower than that of 1 : 2 dienol glucose;

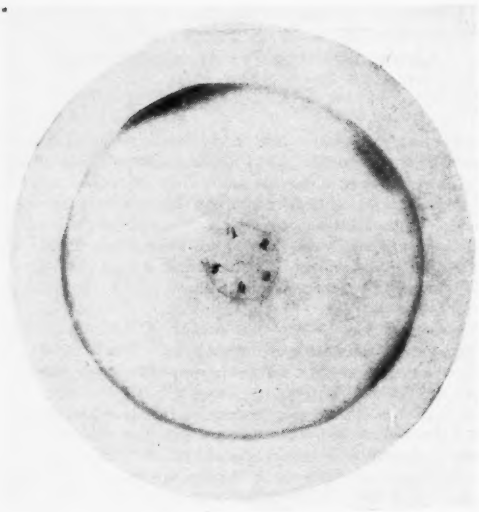

Fig. 1. Circular paper chromatogram of 1 : 2 dienol glucose (2); and unsaponifiable fraction of (1) corn oil; (3) groundnut oil; (5) linseed oil; and (4) dalda (hydrogenated groundnut oil)

it seems probable that prolonged acid hydrolysis of glucose *cyclo*acetoacetate may cause a shift of the double bond of part of the dienol glucose from the 1 : 2 to the 2 : 3 position, and this may be responsible for the second band in the chromatogram.

Hence the *X* factor of the unsaponifiable fraction of crude oils, which Grande *et al.*[4] have suggested is a potent antilipæmic and anti-atherosclerotic factor, would seem to be 1 : 2 dienol glucose, which has already been reported by us to prevent and cure experimental atherosclerosis of various types[7,9].

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Visualization of Acid and Alkaline Phosphatase after Starch-Gel Electrophoresis of Seminal Plasma, Serum and Bile

By starch-gel electrophoresis[1] the acid phosphatase of seminal plasma can be confined to a narrow zone, apparently separated from other proteins in semen[2]. If this technique of separation is to be used for preparative purpose it is, however, necessary to be able to localize the fraction containing phosphatase without disrupting or destroying the gel. The following is a preliminary report on the successful use of a test-paper technique for the visualization of the acid phosphatase fraction. The same method was also used to localize the fractions containing alkaline phosphatase activity after starch-gel electrophoresis of serum and bile.

The test paper was originally described for detection of serum spots in forensic medicine[3]. The paper, which is impregnated with alpha-naphthyl phosphoric acid and diazo-*o*-dianisidin, is easily prepared and stable on storage.

After completion of the electrophoresis a strip of filter-paper is moistened with a suitable buffer (for acid phosphatase an acetate buffer *p*H 5·0, for alkaline phosphatase a borate buffer *p*H 9·8) and is put in contact with the sliced surface of the gel for approximately 30 seconds. The filter-paper is transferred on to a strip of test paper, moistened with the same buffer just before use. In a few seconds or minutes, depending on the activity of the original sample, a purple band appears in the paper strips corresponding to the zone of the gel containing phosphatase. The reaction can be accelerated by incubating the paper strips at 37° C. When samples of low activity are used the coloured band can be developed in a strip of the gel by putting a piece of test paper directly in contact with the gel surface at 37° C. for 1–2 hours.

Using this technique it was possible to locate the zones containing the acid phosphatase and to cut out the corresponding parts of a great number of gels in a short time (*A–B*, Fig. 1). The test paper was also very useful for screening purposes during various steps of purification of the acid phosphatase.

Studies were also made on the localization of the alkaline phosphatase activity after starch-gel electrophoresis of serum and bile. The electrophoresis was carried out in a borate buffer *p*H 10·0 for 17 hr. at 4 V./cm. at room temperature. The serum was obtained from a patient with the diagnosis of choledocholithiasis and showed an alkaline phosphatase activity of 42 Buch-Buch units. The bile was obtained from the autopsy of a patient with severe burns. The main phosphatase activity in the serum (*C–D*, Fig. 1) migrated as a distinct narrow band between the β- and the hapto-globin-fractions. After electrophoresis of some other sera a faint band has also been observed corresponding to the pre-albumin-(acid α_1-) fraction of serum.

The electrophoretic pattern of the bile (*E*, Fig. 1) showed two or possibly three zones of alkaline phos-

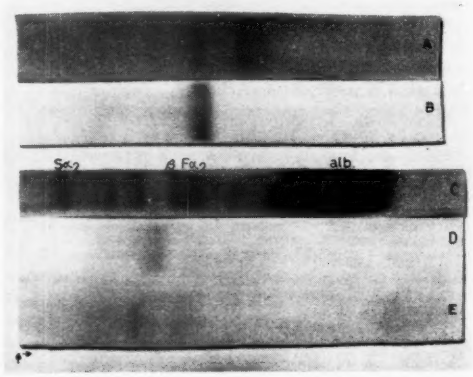

Fig. 1. Starch-gel electrophoresis of seminal plasma (*A–B*), serum (*C–D*) and bile (*E*). *A* and *C* show the protein fractions dyed with amido black, *B*, *D* and *E* the corresponding zones containing acid (*B*) or alkaline (*D–E*) phosphatase activity

phatase activity. The major part of the activity migrated a little slower than that of the serum on the same gel. The same difference in mobility was observed even when albumin in a concentration equal to that in serum had been added to the bile. A weaker zone of activity could be observed corresponding to the pre-albumin- (acid α_1) fraction of serum. When the electrophoresis was carried on for a longer time a third zone of activity close to the origin was observed.

The zones giving a purple colour with the test paper were shown to contain alkaline phosphatase when estimation of the activity[4] were made on eluates of corresponding parts of the gel.

Recently Rosenberg[5] and Keiding[6], using zone electrophoresis on a starch block, have reported on the variation of the alkaline phosphatase activity in the protein fractions of sera from patients with bone and liver diseases. Rosenberg found the phosphatase activity in the serum of normals, and patients with bone disorders and parenchymatous liver diseases in the α_2-region, whereas the serum of patients with obstructive and infiltrative liver diseases also showed an α_1-phosphatase which constituted on an average 16 per cent of the total activity. Keiding noted three zones of activity corresponding to the α_1, α_2- and β-globulin, the first two being increased in liver disorders and the last one in bone diseases. Rosenberg also studied the localization of the phosphatase activity after starch electrophoresis of bile and found that the mobility of a major part of the activity corresponded to the α_1-globulin of serum.

The results of the investigations mentioned above are thus inconsistent with the present observation that in starch gel the main part of serum alkaline phosphatase seems to migrate separately from the major protein fractions. Experiments for isolation of the serum alkaline phosphatase using preparative starch-gel electrophoresis and investigations of the relation between the observed phosphatase fractions of bile and of serum from patients with bone and liver diseases are in progress.

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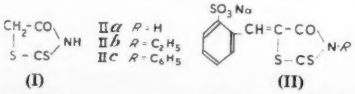
Mothicidal Rhodanine Derivatives

The fungitoxicological properties of a variety of rhodanine derivatives are well established and have been the subject of considerable study[1-3]. In particular, the class of compounds obtained by the condensation of rhodanine (I) or a 3-substituted congener with carbonylic compounds may be conviently used for the investigation of the interrelationships of chemical structure and fungicidal activity.



(I) (II)

The carbonyl component of the condensation has been extensively varied[4-7] and has included aliphatic, aromatic, heterocyclic and polycyclic aldehydes and also ketones, diketones, dialdehydes and trialdehydes. The condensation products have tended, in general, to be complex bulky molecules which concomitantly have been considerably less soluble than their rhodanine precursors in the aqueous media typical of the biological or phytological environments in which they are designed to operate.

In order to obtain water-soluble 5-arylmethylene-rhodanines for evaluation as fungicides, the condensation products of rhodanine and some 3-substituted derivatives with the sodium salts of 2-formylbenzene sulphonic acid and some related benzaldehyde sulphonic acids were prepared[8]. These water-soluble rhodanine-aldehyde condensation products (II) were found to be substantive to wool from a sodium sulphate-sulphuric acid solution. This observation, coupled with the patent disclosures[9] of the mothicidal activity of various mercaptals of 2-formylbenzene sulphonic acid, suggested an evaluation of some examples of (II) in this capacity.

Through the courtesy of Dr. J. Barritt, deputy director of the Wool Industries Research Association, sodium 2-(5′-rhodaninylidenemethyl) benzene sulphonate (II*a*) and its N-ethyl (II*b*) and N-phenyl (II*c*) analogues were tested against the common clothes moth, *Tineola bisselliella* Hummel, using the Association's tube test[10]. The compounds were applied to the wool in a boiling dye-bath in the presence of 3 per cent sulphuric acid and 10 per cent Glauber's salt.

Sodium 2-(5′-rhodaninylidenemethyl) benzene sulphonate was completely ineffective as measured by this test at both the 1 and 5 per cent impregnation levels. Both its N-ethyl (II*b*) and N-phenyl (II*c*) derivatives were, however, effective at a 5 per cent concentration, though the former showed signs of slight attack. The complete protection afforded by sodium 2-(3′-phenyl-5′-rhodaninylidenemethyl) benzene sulphonate (II*c*) was maintained when its concentration in the wool was reduced from 5 to 2 per cent and at the 1 per cent level only an indication of incipient attack was observed. The improvement obtained in mothicidal activity occasioned by variants of the nitrogen substituent in (II) is paralleled in studies of the bacteriotoxic and fungitoxic activity of 3-substituted rhodanines[11].

As far as we are aware the testing of rhodanine derivatives as mothproofing agents has not previously been reported.

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Interaction between Nucleic Acid and a Cationic Detergent

The effect of cetyl-trimethyl-ammonium bromide ('Cetavlon') on the infectivity of Rous sarcoma extracts has already been described by one of us[1,2]. It was suggested that the reversible decrease of the infectivity could be attributed to interaction between the virus nucleic acid and the detergent. It seemed therefore of interest to analyse in detail some aspect of the interaction between 'Cetavlon' and ribonucleic acid.

The precipitation reaction of 'Cetavlon' and ribonucleic acid was studied by turbidimetry in the Beckmann spectrophotometer at 580 mμ, or, better, by determination of the optical density at 260 mμ of the supernatants after 60 min. centrifugation at 18,000*g*. Preliminary experiments showed that several commercial preparations of ribonucleic acid contain variable amounts of dialysable material, absorbing at 260 mμ, which could not be precipitated by 'Cetavlon'. The preparations used in the present investigation were therefore further purified by precipitation with alcohol or 'Cetavlon'.

Fig. 1 shows the pattern of the precipitation of a fixed quantity of ribonucleic acid (50 μgm./ml., in water, at 20° C., final *p*H 7) by increasing amounts of 'Cetavlon'. The results have been reported as per cent ribonucleic acid precipitation versus the weight-ratio 'Cetavlon' ribonucleic acid. They clearly indicate that the maximum precipitation is obtained for a detergent/ribonucleic acid ratio of 1–2: outside the region of maximum precipitation the curve falls steeply.

The ratio 'Cetavlon' ribonucleic acid for optimum precipitation appears to depend somewhat on the absolute concentration of the ribonucleic acid, going from 1 to 3. The precipitation of ribonucleic acid by 'Cetavlon' is strongly affected by the salt concentration of the medium. Fig. 2 shows the quantity of ribonucleic acid in the supernatant after centrifugation, for a 'Cetavlon'/ribonucleic acid ratio of 2, for different concentration of salts in the medium.

It is clear that the amount of ribonucleic acid in the supernatant increases with the salt concentration and that the effect varies with different salts. Urea and glucose are without appreciable effect.

In the presence of a small salt concentration the precipitation of ribonucleic acid as a function of the 'Cetavlon'/ribonucleic acid ratio shows a pattern different from that observed in water; particularly as regards the zone of detergent excess (Table 1).

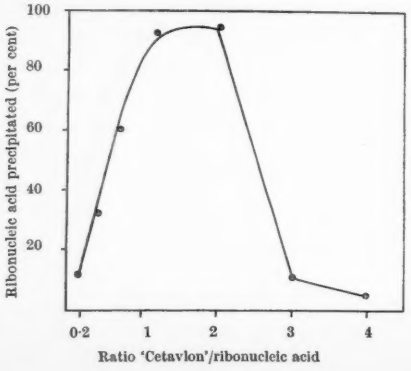

Fig. 1

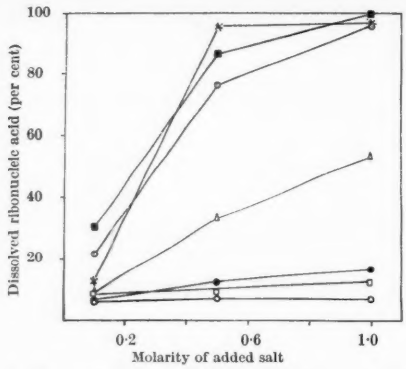

Fig. 2. Per cent of dissolved ribonucleic acid for a reaction 'Cetavlon' + ribonucleic acid ('Cetavlon'/ribonucleic acid ratio = 2) as function of molarity of added salt. ✱—✱, sodium chloride; ○—○, sodium sulphate; ●—●, glycine; ■—■, sodium citrate; △—△, sodium phosphate; □—□, glucose; ○—○, urea

Table 1. Influence of Sodium Chloride on the Amount of Ribonucleic Acid (RNA) (in per cent) in Supernatants of 'Cetavlon'-Ribonucleic Acid Mixtures after Centrifugation

'Cetavlon'/RNA	Water	8×10^{-2} *M* NaCl
0·2	89·2	91·5
0·4	69·0	72·0
0·8	41·2	42·0
1·6	6·8	8·3
2	5·6	3·7
3	89·0	4·3
4	95·0	7·7

In salt solutions the precipitation is not affected significantly by *p*H between *p*H 5 and 9, but on both sides of these *p*H's the amount of ribonucleic acid which can be precipitated by the 'Cetavlon' decreases considerably with increase of acidity or basicity.

There is evidence that the precipitate appearing after the interaction of ribonucleic acid and 'Cetavlon' is due to the formation of an insoluble complex. This complex (obtained by mixing ribonucleic acid and 'Cetavlon' in Miller and Golder buffer at *p*H 7·0, 0·1 ionic strength) was collected by centrifugation, repeatedly washed, and dissolved in 1·0 *M* sodium chloride. It contained about 30 per cent ribonucleic acid by weight.

The precipitation reaction between 'Cetavlon' and ribonucleic acid is of interest as a model for the study of the interaction of detergents with virus particles.

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Inhibition of Liver Hexokinase by Dehydroascorbic Acid and Alloxan

The structure and properties of dehydroascorbic acid are to a certain extent similar to those of alloxan, and both the substances are diabetogenic in rats[1,2]. It was previously reported by Bhattacharya, Robson and Stewart[3] that dehydroascorbic acid causes a precipitous but temporary fall in the reduced gluta-

thione content of rat liver. Their results suggested that the fall in reduced glutathione was not due to oxidation but due to a complex formation between the sulphydryl group of glutathione and dehydroascorbic acid. Hexokinase is an enzyme the activity of which probably depends on the presence of free sulphydryl group(s)[4,5]. It seemed desirable, therefore, to study the effect of dehydroascorbic acid on the hexokinase activity of rat liver homogenate. Although Griffiths[6] reported that cysteine is capable of releasing the inhibition of muscle hexokinase by alloxan, in view of the results obtained with dehydroascorbic acid it was decided to study the effect of alloxan and cysteine on liver hexokinase.

Rat liver was homogenized according to the method of Crane and Sols[7]. The homogenate was centrifuged for 30 min. at 12,000 r.p.m., and the clear pink supernatant was used, without further treatment, as the source of enzyme. All the above operations were carried out at − 2° C., and the supernatant was stored at this temperature until used. Enzyme preparations (0·4 ml.) were incubated for 30 min. at 30° C. with 0·03 molar *tris*(hydroxymethyl)-aminomethane buffer containing 0·003 molar ethylenediaminetetraacetic acid and 0·003 molar magnesium chloride (*p*H 8·0), 0·005 molar glucose, 0·004 molar magnesium chloride, 0·0075 molar adenosine triphosphate, and 0·05 molar sodium fluoride in a total volume of 2 ml. The enzyme activity was measured by the acid-labile phosphate disappearance method[7]. Concentrated solution of dehydroascorbic acid[3] was diluted to give the required concentration. Alloxan solution was prepared immediately before use. When the enzyme was assayed in the presence of dehydroascorbic acid or alloxan, which lowered the *p*H of the reaction mixture by approximately 0·5 unit, the *p*H of the control sample was lowered to the same extent by the addition of dilute hydrochloric acid (0·05 *N*). Dehydroascorbic acid or alloxan was allowed to react on the enzyme for 5 min., in presence of the buffer and sodium fluoride, either before the addition of cysteine hydrochloride or in its presence. Cysteine hydrochloride solution was carefully neutralized to *p*H 7·2 with 1 *N* sodium hydroxide. The *p*H of alanine solutions was adjusted to 7·3 with the 0·03 *M* *tris* buffer.

The enzyme is inhibited to the extent of 30–75 per cent by 1×10^{-3} *M*–$5{\cdot}74 \times 10^{-3}$ *M* dehydroascorbic acid (Fig. 1). At equimolecular concentration ($2{\cdot}87 \times 10^{-3}$ *M*), alloxan is a more powerful inhibitor than dehydroascorbic acid (Table 1). Table 1 shows

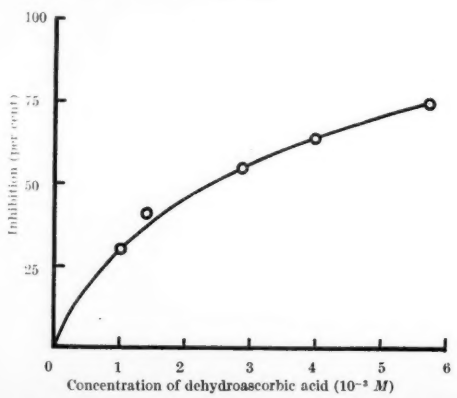

Fig. 1. Inhibition of rat liver hexokinase by dehydroascorbic acid

Table 1. Effect of Dehydroascorbic Acid, Alloxan, Cysteine, Alanine and Hydrochloric Acid on the Activity of Liver Hexokinase

No. of Experiments	Sample	Additions	Disappearance of phosphate after 30 min. (μ moles)	Inhibition (per cent)
4	Control	HCl	4·7	—
	Exp.	$2{\cdot}87 \times 10^{-3}$ *M* DHA*	2·1	55
3	Control	HCl + 5×10^{-3} *M* cysteine	4·9	—
	Exp.	$2{\cdot}87 \times 10^{-3}$ *M* DHA + 5×10^{-3} *M* cysteine	2·3	53
	‡Exp.	5×10^{-3} *M* cysteine + $2{\cdot}87 \times 10^{-3}$ *M* DHA	2·3	53
4	Control	HCl + $2{\cdot}87 \times 10^{-2}$ *M* cysteine	5·1	—
	Exp.	$2{\cdot}87 \times 10^{-2}$ *M* DHA + $2{\cdot}87 \times 10^{-2}$ *M* cysteine	2·2	57
	‡Exp.	$2{\cdot}87 \times 10^{-2}$ *M* cysteine + $2{\cdot}87 \times 10^{-2}$ *M* DHA	4·7	8
2	Control	HCl + $2{\cdot}87 \times 10^{-2}$ *M* alanine	5·2	—
	Exp.	$2{\cdot}87 \times 10^{-2}$ *M* DHA + $2{\cdot}87 \times 10^{-2}$ *M* alanine	2·3	56
	‡Exp.	$2{\cdot}87 \times 10^{-2}$ *M* alanine + $2{\cdot}87 \times 10^{-2}$ *M* DHA	2·3	56
3	Control	HCl	5·0	—
	Exp.	$2{\cdot}87 \times 10^{-3}$ *M* alloxan	1·1	78
2	Control	HCl + 5×10^{-3} *M* cysteine	4·2	—
	Exp.	$2{\cdot}87 \times 10^{-3}$ *M* alloxan + 5×10^{-3} *M* cysteine	1·1	74
	‡Exp.	5×10^{-3} *M* cysteine + $2{\cdot}87 \times 10^{-3}$ *M* alloxan	1·2	71
3	Control	HCl + $2{\cdot}87 \times 10^{-2}$ *M* cysteine	6·7	—
	Exp.	$2{\cdot}87 \times 10^{-2}$ *M* alloxan + $2{\cdot}87 \times 10^{-2}$ *M* cysteine	1·6	76
	‡Exp.	$2{\cdot}87 \times 10^{-2}$ *M* cysteine + $2{\cdot}87 \times 10^{-2}$ *M* alloxan	5·7	15
2	Control	HCl + $2{\cdot}87 \times 10^{-2}$ *M* alanine	5·1	—
	Exp.	$2{\cdot}87 \times 10^{-2}$ *M* alloxan + $2{\cdot}87 \times 10^{-2}$ *M* alanine	1·0	80
	‡Exp.	$2{\cdot}87 \times 10^{-2}$ *M* alanine + $2{\cdot}87 \times 10^{-2}$ *M* alloxan	1·0	80

* DHA = dehydroascorbic acid.
‡ In these experiments dehydroascorbic acid and alloxan were added in presence of cysteine or alanine.

that 5×10^{-3} *M* cysteine and $2{\cdot}87 \times 10^{-2}$ *M* alanine, when added before or after the addition of $2{\cdot}87 \times 10^{-3}$ *M* dehydroascorbic acid and alloxan, failed to protect or reactivate the enzyme. Once the enzyme was inhibited by $2{\cdot}87 \times 10^{-3}$ *M* dehydroascorbic acid and alloxan, even the addition of $2{\cdot}87 \times 10^{-2}$ *M* cysteine had no effect. On the other hand, when the above concentration of dehydroascorbic acid or alloxan was added in the presence of $2{\cdot}87 \times 10^{-2}$ *M* cysteine, the enzyme was protected to a significant extent. Virtually the same results were obtained when the above experiments were repeated in presence of glucose and adenosine triphosphate in the reaction mixture.

It is clear from these results that the enzyme is irreversibly inhibited by dehydroascorbic acid and alloxan. Although the irreversibility of the reaction may be explained by the formation of stable addition compounds between the functional sulphydryl group(s) of the enzyme and the diabetogenic substances, it is possible that a more complex mechanism is involved. It is almost certain, however, that the inactivation is not due to simple oxidation of the sulphydryl group(s) of the enzyme; if this were so, it would have been possible to release the inhibition by cysteine. Griffiths, on the other hand, reported that cysteine can reactivate alloxan-inhibited muscle hexokinase[6]. It seems that muscle hexokinase differs in this respect from liver hexokinase.

The protective action of cysteine was apparent when its concentration was ten times greater than those of dehydroascorbic acid and alloxan. This is not surprising since only large amounts of exogenous cysteine can protect rats from the diabetogenic action of dehydroascorbic acid and alloxan[2]. The fact that the enzyme is protected by cysteine, but not by alanine, indicates that the sulphydryl group of cysteine is responsible for inactivating dehydroascorbic acid and alloxan.

I wish to thank Prof. F. C. Happold for laboratory facilities, and the University of Leeds for an Imperial Chemical Industries Fellowship.

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Chromatographic Fractionation of Rat Tail Tendon Collagen

Physical studies[1] indicate that solubilized collagens give rise to two components when heated gently (13°–40° C. depending on the source of the collagen)[2] or when treated with other agents which break weak bonds. These two components have not been satisfactorily isolated[3] by the methods used to fractionate[4] 'gelatine', an end-product of more drastically altered collagen. We have succeeded in chromatographing warm dissolved rat tail tendon collagen into four components with a method which allows for the isolation of these components.

Tail tendons were obtained from Walter Reed Albino rats, weighing 300 ± 5 gm. The tendons were washed overnight at 4° C. in 0·2 *M* sodium chloride, rinsed in distilled water, immersed in measured volumes of acetic acid, and allowed to stand at room temperature for 24 hr. The mixtures were filtered through medium sintered glass funnels (pore size 10–15 micra) after high-speed centrifugation, yielding clear viscous solutions. The chromatography was done with jacketed columns kept at 40° C. by circulating water. The columns measured 0·9 cm. in diameter and were poured to a height of 7·0 cm. from a slurry made of carboxymethyl cellulose[5] and 0·2 *M* sodium hydroxide and 0·5 *M* sodium chloride. The gradient elution arrangement consisted of a separatory funnel reaching to the bottom of a side-arm flask which was stirred. The critical parameters of the gradient elution system were (1) concentrations of the lower solution— 0·05*M* sodium chloride, 0·05 *M* sodium dihydrogen phosphate, and of the upper solution—0·14 *M* sodium chloride, 0·05 *M* sodium dihydrogen phosphate, and (2) the volume of the lower flask up to its side-arm, 160 ml. 1·5 ml. of sample containing 45 mgm. per cent nitrogen were allowed to sink into the adsorbent which had been equilibrated with the lower solution, washed with distilled water and allowed to dry. A modification of the Folin method[6] was used to spot the effluent fractions which were collected at flow rates of 20 ml. per hour. A standard micro-Kjeldahl procedure was used to measure the total nitrogen in the identified peaks.

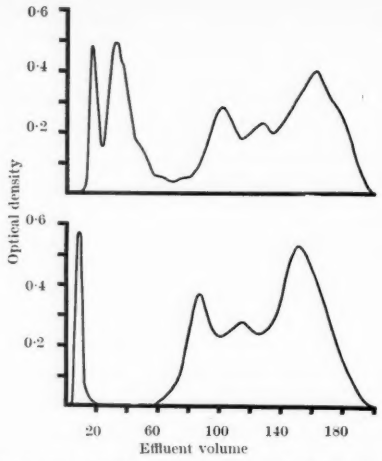

Fig. 1. Top, 0·6 *M* acetic acid ; bottom, 0·1 *M* acetic acid

All the chromatograms of solutions made with acetic acid ranging from 0·005 *M* to 1·0 *M* consist of at least 4 components (Fig. 1): three incompletely separated peaks found in the latter half of each chromatogram, called fractions 2, 3 and 4; and one or more peaks preceding these, collectively labelled fraction 1. Fraction 1 nitrogen makes up less than 1 per cent of the total at 0·05 *M* ; this percentage rises rapidly above 0·25 *M* so that fraction 1 contains 88 per cent of the total nitrogen at 1·0 *M*. This is associated with a proportional fall in fractions 2, 3 and 4. The hydroxyproline content of fraction 1 at 0·05 *M* is very low (0·7 per cent); it, too, rises sharply above 0·25 *M* almost attaining the levels of the unfractionated solutions of equivalent acid concentration. In fractions 2 + 3 + 4, the hydroxyproline to nitrogen ratio approximates that of the unfractionated solutions at all concentrations. The fractions 1, poor in hydroxyproline, obtained from acetic acid solutions below 0·25 *M* may represent the 'youngest' portions of the collagen structure; they appear to be loosely bound segments since they could be obtained from unwarmed fractions which was impossible for fractions 2, 3 and 4. Above 0·25 *M* the bulk of the collagen was altered dramatically at 40° C., giving rise to fractions 1 with normal amounts of hydroxyproline.

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Intracellular Thioctic Acid and Coenzyme A following Vanadium Treatment

It was suggested in a previous communication[1] that the fall in coenzyme A liver concentrations in animals treated with vanadium might be due, at least partially, to a reduction in the number of —SH groups available. This hypothesis was based on the following facts: (1) The toxic effects of vanadium may, to a great extent, be attributed to modifications in reactions connected with sulphur compounds, especially those of the thiolic type[2]. (2) The lack of —SH groups, and particularly of sulphurated amino-acids, lowers the coenzyme A content in the liver[3]; in this case, a fall in thioctic acid, the connexion of which with coenzyme A is well-known, also occurs in the tissues[4].

These investigations were undertaken on the basis of the above considerations to assess the effects of vanadium treatment on the liver content and intracellular distribution of thioctic acid and coenzyme A.

The experiments were performed on rats bred by us, of average weight 120–150 gm., fed on a diet of known composition[1] starting 7 days after the beginning of the experiment. Vanadium was administered mixed with their diet, at the level of 0·05 per cent, in the form of sodium metavanadate ($NaVO_3 \cdot 4H_2O$) for 14 days. At the end of this period, the animals were killed by bleeding, and their livers removed for analysis.

Some analyses were carried out on the whole liver homogenate, and some on fractions obtained by centrifugation at 500 r.p.m. (nuclei), 3,000 r.p.m. (mitochondria) and 20,000 r.p.m. (microsome particles), in accordance with the standard procedure.

The following determinations were then made: coenzyme A[5]; thioctic acid[6]; and proteins[7]. The results are given in Table 1.

In the whole homogenate more thioctic acid disappeared than coenzyme A; the sharpest reduction occurred in the mitochondrial fraction, where thioctic acid and coenzyme A were respectively 86 and 52·5 per cent below normal; in the remaining homogenate fractions, variations in both thioctic acid and coenzyme A, were far less marked than those observed in mitochondria.

These results would suggest a slow-down in the biosynthesis of coenzyme A, caused, at least in part, by the non-decarboxylation of pantothenylcysteine to pantotheine, which process is known to require pyridoxal phosphate as the prosthetic group: indeed, vanadium would seem to affect this reaction[8].

The fall of thioctic acid concentrations in the tissues might be regarded partly as a clue to a more widespread alteration in the metabolism of sulphurated substances, and partly as the result of the lack of coenzyme A, which is essential for restoring the dithiolic form of the lipothiamide.

In other words, the fall in coenzyme A concentrations in the livers of animals treated with vanadium might be the result of two different actions exerted (1) on pyridoxal-phosphate and, (2) on the metabolism of sulphurated substances. This would appear to be confirmed by the preliminary results of investigations now being performed in this Laboratory; treatment with pantothenic acid and/or cysteine causes only a slight increase of coenzyme A concentrations in the liver of rats kept on a diet supplemented with vanadium, so a substantial degree of protection appears to be provided by pantotheine.

Table 1. Thioctic acid and coenzyme A in liver homogenate fractions from animals treated with vanadium (average values ±S.E.)

Homogenate Fraction	Thioctic Acid: control (8)*	μgm./gm. Protein: treated (10)	Coenzyme A: control (8)	U/100 mgm. Protein: treated (10)
Whole	8·33±0·39	2·37±0·18	91·6± 4·7	50·8±3·8
Mitochondria	29·37±1·64	4·16±0·25	260·6±11·5	124·1±8·6
Nuclei	4·98±0·18	3·58±0·20	67·1± 3·1	59·8±5·0
Microsome	—	—	23·3± 1·8	11·0±0·9
Supernatant	4·57±0·21	2·44±0·13	51·8± 2·4	35·4±2·7

* Number of animals given in brackets.

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Deaminases of Adenylic Acid and Adenosine in Rat Kidney

Hitherto it has been generally accepted that only muscle, blood and nervous tissue contain a specific adenylic deaminase[1,2]. Recently, however, we have succeeded in separating adenylic deaminase from adenosine deaminase by using rat kidneys.

Rat kidney homogenate prepared in 0·1 *M* potassium chloride containing 0·039 *M* borate buffer *p*H 7, produced both ammonia and inorganic phosphate when incubated with muscle adenylic acid. When the homogenate was fractionated by differential centrifugation, the phosphatase activity was localized mainly in the mitochondrial fraction (sedimenting between 3,000 and 16,000 *g*). This fraction, however, was practically devoid of deaminase activity (Table 1). After the centrifugation of the mitochondrial fraction there remained a clear reddish liquid (cytoplasm). When dialysed against distilled water at 0°C. for 24 hr., the cytoplasm separated into a water-insoluble fraction (cytoplasmic globulins) and a water-soluble

Table 1. Deamination and Dephosphorylation of Adenylic Acid by Rat Kidney Fractions

Fraction present in the incubation mixture		μmole P/mgm. protein-N	μmole NH_3/mgm. protein-N
	a	4·42	3·42
Whole homogenate	*b*	3·23	1·89
	c	3·98	2·58
	a	14·70	1·71
Mitochondrial fraction	*b*	7·23	0·0
	c	8·85	0·80
	a	1·40	2·68
Cytoplasmic albumins	*b*	1·33	0·56
	c	1·05	1·11
	a	1·21	5·65
Cytoplasmic globulins	*b*	1·43	5·45
	c	1·82	7·87

Rat kidneys were homogenized in 7·5 vol. of 0·1 *M* potassium chloride containing 39 m*M* borate buffer *p*H 7, the homogenate centrifuged at 3000 *g* for 10 min., and the sediment disregarded. The supernatant was centrifuged at 16000 *g* for 15 min. The sediment thus obtained is called the 'mitochondrial fraction'. The clear supernatant solution was further separated in two fractions by dialysis against distilled water for 24 hr.: the water-soluble 'cytoplasmic albumins', and the water-insoluble 'cytoplasmic globulins'. The letters *a*, *b*, *c*, indicate different animals.

The incubation mixtures contained 0·025 *M* succinate buffer *p*H 6, 0·005 *M* adenylic acid, 0·1 *M* potassium chloride and the appropriate kidney cell fraction. Incubations were terminated after 30 min. at 25°C. by the addition of trichloroacetic acid to the final concentration of 7·5 per cent. In the filtrates inorganic phosphate was estimated by the method of Gomori (ref. 3) and ammonia according to Russel (ref. 4) by Conway's microdiffusion method. The results, each representing an average from two samples obtained from one animal, were expressed in terms of μmoles/mgm. protein-nitrogen.

Table 2. The Localization of Adenylic Deaminase and Adenosine Deaminase in Rat Kidney Fractions

Fractions present in the incubation mixture		μmole P	μmole NH_3
Mitochondrial fraction	*a*	4·11	0·48
	b	2·02	0·00
	c	2·18	0·20
Cytoplasmic albumins	*a*	0·35	0·67
	b	0·24	0·10
	c	0·19	0·20
Cytoplasmic globulins	*a*	0·35	1·64
	b	0·40	1·53
	c	0·40	1·73
Cytoplasmic albumins + mitochondrial fraction	*a*	4·52	2·60
	b	2·50	1·63
	c	2·66	1·73
Cytoplasmic globulins + mitochondrial fraction	*a*	4·00	2·03
	b	2·42	1·43
	c	2·50	1·63

Fractions and conditions of incubation were as described in Table 1. The results are expressed as μmoles of phosphorus and ammonia, liberated in mixtures (2 ml.) containing either 0·3 ml. of a single fraction or 0·3 ml. of the cytoplasmic fraction plus 0·3 ml. of the mitochondrial fraction.

fraction (cytoplasmic albumins). Table 2 shows that the globulin fraction deaminated adenylic acid readily, whereas the cytoplasmic albumins produced ammonia from adenylic acid only when the mitochondrial fraction had been added to it, indicating that in the latter case, adenylic acid was first dephosphorylated to adenosine, and this afterwards deaminated.

The conclusion is that the globulin fraction contains mainly the adenylic deaminase while the albumin fraction contains the adenosine deaminase; this has been confirmed by experiments in which the two cytoplasmic fractions were incubated with adenylic acid or adenosine and the reaction mixtures analysed by paper chromatography. It can be seen from

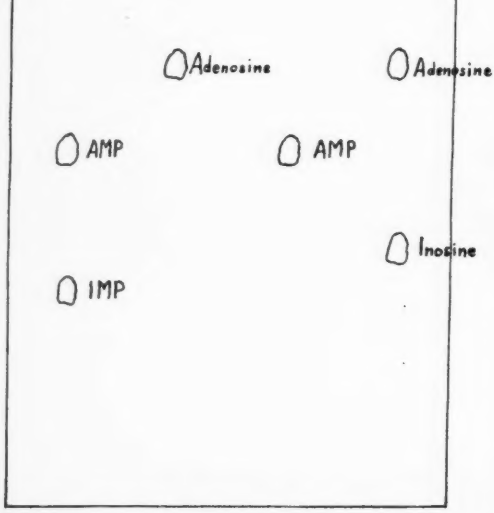

Fig. 1. The result of incubation of cytoplasmic fractions with adenosine and with adenylic acid as investigated by paper chromatography.
I, Cytoplasmic globulins incubated with adenosine monophosphate
II, Cytoplasmic globulins incubated with adenosine
III, Cytoplasmic albumins incubated with adenosine monophosphate.
IV, Cytoplasmic albumins incubated with adenosine
Descending chromatography on Macherey—Nagel *S*.61 filter paper in a solvent system: saturated ammonium sulphate: isopropanol: water/79:2:19 according to Deutsch and Nilsson (ref. 5) was carried out, after trichloroacetic acid had been extracted with ether from the reaction mixture.

Fig. 1 that sample I in which the globulin fraction reacted with adenylic acid, produced inosinic acid, whereas sample II which contained the same fraction and adenosine, was incapable of deaminating adenosine. On the other hand, when the albumin fraction was incubated with adenylic acid (sample III) there appeared only one spot, corresponding to unchanged adenylic acid. However, adenosine and inosine could be identified on the chromatogram when the albumin fraction has been incubated with adenosine (sample IV).

As yet our understanding of the physiological role of the enzymes deaminating adenine derivatives is incomplete; but it is evident from the present study that the enzyme which deaminates adenylic acid is more widely distributed in animal tissues than has been hitherto supposed.

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Preparation of a Highly Purified Carbohydrate-free Johnin Protein

The preparation of a complement-fixing antigen of carbohydrate nature from *Mycobacterium johnei* has been described in a previous publication[1]. The present communication deals with the isolation and purification of a carbohydrate-free protein antigen from Johne's bacilli killed with phenol. This antigen has been shown to be active in complement fixation tests with sera of certain animals immunized with johnin purified protein derivative in adjuvants. Heat-killed organisms are not suitable for the preparation of the purified specific protein since distintegration products, formed at these relatively high temperatures, interact to form complexes difficult to separate. However, a 'cold method', based on urea extraction, proved to be practical. This relatively simple procedure yielded fair amounts of a protein devoid of serologically-active carbohydrates and of nucleic acid contaminants.

The strains of Johne's bacilli used in the assays and the method of their culture were similar to those referred to in the paper mentioned above[1] and do not need to be repeated here. The preparation of the specific antigen is as follows. After a three-month growth period, the phenol-killed and filtered bacilli are defatted by acetone, dried by aeration at room temperature and then milled to a fine powder. The powder is suspended in a proportion of 1 : 10 in a 6 *M* urea solution, alkalinized slightly with 0·4 *M* potassium bicarbonate, and this is stirred overnight in the cold. The dense suspension is separated by Sharple's centrifugation into a yellow viscous supernatant, which is decanted, and a sediment, which is discarded. Addition of 4 per cent trichloracetic acid to the supernatant precipitates the crude antigen which is allowed to coalesce for some time at room temperature before spinning it down. The centrifugate, after being washed once with 1 per cent trichloracetic acid, is redissolved in a moderate volume of distilled water at *p*H 7·2–7·5 by the addition of sodium hydroxide. For clarification the solution has to be centrifuged at high speed. In order to remove diffusible impurities it is dialysed against several changes of distilled water for 4 days in the cold.

If the solution is clear after dialysis no further centrifugation is necessary and the protein can be precipitated immediately at *p*H 3·8 by the addition of *N* hydrochloric acid. The precipitate is collected by centrifugation, suspended in alcohol-ether (3 : 1), washed and dried with absolute alcohol and anhydrous ether. 100 gm. of the bacillary powder, corresponding to 500 gm. of the drained, wet bacilli yielded an average of 6–7 gm. protein. Neutral distilled water or saline solutions of the antigen can be prepared by addition of 0·1 *N* sodium hydroxide. Although the almost colourless product is free of serologically reactive carbohydrates, it is still markedly contaminated with nucleic acids. Moving boundary electrophoresis carried out on a 1 per cent solution in veronal buffer at *p*H 8·6, ionic strength 0·1, shows a pattern of three peaks. For additional purification 5 per cent crystalline trichloracetic acid is added to a 2 per cent solution of the preparation. The separated precipitate is incubated in the acidic solution for 1 hour at 70° C. Then the precipitate is collected by centrifugation, washed once with 1 per cent trichloracetic acid and several times with alcohol-ether (3 : 1) and dried with alcohol and ether. While the product still maintains its serological reactivity it does not contain spectrophotometrically detectable nucleic acid residues and shows only a single peak in its electrophoretic pattern. This, however, can be split into two sub-units after incubation with 1 per cent crystalline pepsin at *p*H 3·0 for six minutes at room temperature[2] as shown in Fig. 1.

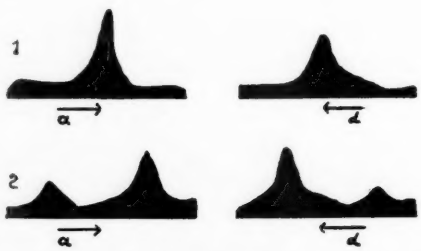

Fig. 1. Electrophoretic pattern of 1 per cent purified Johnin-protein antigen before pepsin incubation, in veronal buffer at *p*H 8·6, ionic strength 0·1 (ref. 1), incubated with pepsin in a ratio 1 : 100 at room temperature at *p*H 3·0 for 6 minutes (ref. 2)

In preliminary comparative intradermal tests on sensitized guinea pigs this purified protein appeared of a higher potency than a johnin purified protein derivative prepared by standard methods and was equal in potency to another purified protein derivative that had been freed of residual impurities by dialysis. Five intradermal injections of the protein antigen in ten guinea pigs, at bi-weekly intervals did not sensitize them to any noticeable degree.

The co-operation of Miss L. Duhamel in testing this fraction for complement fixing activity, of Dr. C. E. Rice for discussing results, and of Dr. H. Konst in carrying out and evaluating the biological test is gratefully acknowledged. Thanks are due to Miss M. M. Schingh for the preparation of the bacterial cultures and to Mr. J. A. G. Larose for his valuable technical asistance.

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PHYSIOLOGY

Hæmodynamics in Essential Hypertension

According to present-day concepts the raised blood pressure of essential hypertension is thought to be due to a more or less parallel increase in vascular resistances in all the important regions of the body, while cardiac output remains unchanged[1]. This view is based on measurements of flow of blood through the whole upper extremity[2] or through its skin[3], which gave values not exceeding those of normotensive subjects, as would be expected should the rise in blood pressure be due to a localized increase of resistance, for example, in the splanchnic area. However, flow of blood in skin and muscle might be changed in opposite directions, and, therefore, such isolated investigations might give misleading information in this respect.

We have, therefore, re-examined this problem in 12 healthy normotensive subjects and in 15 patients suffering from essential hypertension. None of them had any clinical signs of heart failure. All the studies were carried out in the morning, under fasting resting conditions. Blood pressure was registered directly from a percutaneously cannulated brachial artery. Cardiac output was estimated by the dye dilution technique, renal plasma flow by *PAH* clearance. Splanchnic hæmodynamics were assessed from the disappearance curve of rose bengal[4]. Flow of blood in the skin (on the volar side of the forearm) was measured with the 'Wärmeleitmesser' of Hensel and Bender[5], that of muscle of the forearm was estimated from the difference between forearm blood flow (measured by occlusion plethysmography) and blood flow through the corresponding skin area. From blood pressure and blood flow regional resistances were calculated.

The results are summarized in the first line of Fig. 1. The average hæmodynamic data of the hypertensives are expressed in percentage of the averages of the normotensive group, and the differences were evaluated statistically by Student's *t* test. In subjects with essential hypertension there is a significant increase in vascular resistance in the kidneys, in the splanchnic area and in the skin, and a marked decrease of vascular resistance in the forearm muscles.

This result differs from the present view and demonstrates that, in essential hypertensives, blood is shifted from the viscera and skin to muscle. This hæmodynamic change is analogous to that produced by an acture mental stress acting as a pressor stimulus in normotensive subjects[6], which is summarized in the second line of Fig. 1. The average changes of individual functions are expressed as percentage of the resting values of the same group.

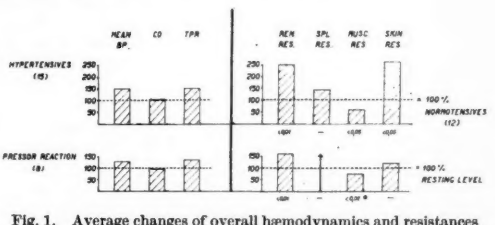

Fig. 1. Average changes of overall hæmodynamics and resistances in the leading vascular areas in subjects with essential hypertension and in normal subjects under mental stress. Horizontal dotted line, set at 100 per cent indicates the control level of normotensives (line 1) and the resting level of normotensive subjects under investigation (line 2). The arrow in the column of splanchnic resistance indicates the overall trend of results which were not numerous enough for statistical evaluation.

The change of the total peripheral vascular resistance in the acute pressor reaction depends on the mutual balance of visceral vasoconstriction and muscular vasodilatation. When the latter prevails, total peripheral vascular resistance drops, and vice versa. Cardiac output increases in all these instances where total peripheral vascular resistance either decreases, or—due to a perfect balance of visceral vasoconstriction and muscular vasodilatation—remains unchanged. Cardiac output tends to decrease whenever total peripheral vascular resistance markedly increases[6]. It is obvious from Fig. 1 that in our group of essential hypertensives, cardiac output during rest did not differ from its average level in the normotensives, while total peripheral vascular resistance was markedly higher. This is in accord with the findings of others[7,8] and demonstrates that the hæmodynamic state which we have investigated corresponded to the overall hæmodynamic pattern of essential hypertension. At the same time this overall hæmodynamic change of hypertension is analogous to that found in acute pressor reactions, when visceral vasoconstriction prevails over muscular vasodilatation.

While the acute pressor reactions in normal people have a tendency to subside promptly after cessation of the pressor stimulus, they are not only exaggerated[9] but also very protracted[10] in hypertensives and in some normotensives ('hyperreactors'). The analogy between the identical detailed hæmodynamic pattern of these pressor reactions and between the hæmodynamic conditions in essential hypertension at rest lends support to the suggestion that the hæmodynamics of essential hypertension are a fixation of the hæmodynamic state produced by stressful stimuli in normotensive subjects.

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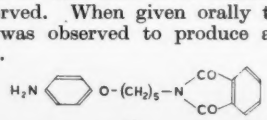
Loss of Wool in Sheep caused by l-*p*-Aminophenoxy-5-phthalimidopentane

Some striking toxic effects have been described for certain antibilharzial compounds[1,2,3]. These consist of visual impairment and loss of hair in laboratory animals. More recently[4], the activity and toxicity of some N-*p*-aminophenoxy-alkylamides have been described in experimental schistosomiasis. One of these compounds*, 1-*p*-aminophenoxy-5-phthalimidopentane, (M and B 2948*A*)(I), has been tested for its effect against liver fluke in sheep and the purpose of this note is to describe the toxic effects observed. When given orally to sheep this compound was observed to produce a remarkable loss of wool.



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Crossbred ewe and wether lambs, 5–8 months old and weighing between 40 and 124 lb., were used. They were kept on pasture and each, with the exception of the black sheep, had been infected experimentally with 200 metacercariæ of *Fasciola hepatica* obtained from a laboratory colony of infected *Limnæa truncatula*. M and B 2948*A* was given as a drench using a 20 per cent ball-milled suspension.

Seven infected sheep were used in all. Initially a ewe lamb was given M and B 2948*A* at 1 gm./kgm. body-weight, and returned to pasture. Six days later the animal appeared to be losing wool, and this came out in handfuls when the ewe was caught. On the ninth day after dosing the lamb was almost denuded of wool with the exception of the parts of the body covered with hair, but no other ill-effects were observed. A wether lamb which received 2 gm./kgm. body-weight started to lose wool after two days, and was naked except for head and leg hair after seven days. Loss of wool occurred in three other lambs given 2·0, 0·5 and 0·25 gm./kgm. body-weight, respectively, but no loss of wool was observed in two lambs given 0·125 and 0·0625 gm./kgm.

Following the loss of wool, the skin felt moist, and weals were produced when the skin was bruised or scratched. The shed wool had yellow moist 'secretions' adhering to the base of the fibres. Histological examination of skin taken from areas from which the wool had been lost showed many empty follicles. A few scabs and sores which appeared on the naked sheep approximately 10 days after treatment disappeared as the new wool started to grow. An even growth of new wool appeared on the body, and this wool was $\frac{1}{4}$–$\frac{1}{2}$ in. long about one month later. Sheep which did not receive the drug and which were kept on the same pasture lost no wool.

As the pigmented hair on the legs and face appeared unaffected, the possibility was considered that lack of pigment in the wool may be a factor concerned in causing wool loss. Two black-wooled sheep, one a ewe and the other a wether, were given 2 and 1 gm./kgm. body-weight respectively. After six days both were losing wool and this continued until eventually both sheep became almost denuded, with the exception of the hair on the face and legs.

The effect of M and B 2948*A* in young Ayrshire calves was tried. These animals, which had recovered from a *Babesia bovis* infection and which had previously been splenectomized by my colleague, Mr. J. M. S. Lucas, were dosed orally with M and B 2948*A* at 1 gm./kgm. body-weight. One of the calves was observed for ten days and the remaining two for approximately five weeks. At the end of this period no noticeable loss of hair had occurred.

It is of interest to note that Goodwin, Richards and Udall[2] observed loss of hair in mice two or three weeks after administering two or three large doses of a related compound, but the vibrissæ and claws were not affected. They also noted that the fur remaining became changed in texture. This change of texture also occurred in rats, but no loss of hair was observed in these animals, rabbits, cats or monkeys. They considered that the drug probably affected the follicles, which failed to develop, leaving bald patches when the older hairs had been shed. Subsequent crops of follicles were not affected and six weeks after treatment the hair had grown again.

The marked wool-shedding in sheep described in this note occurred within a few days of the administration of M and B 2948*A* as a single dose but no effect was observed on the hair of the face and lower leg regions. The hair of calves was not noticeably

affected. My colleague Hill[5] did not observe any loss of hair with M and B 2948*A* when administered to mice, rats, rabbits, guinea pigs, hamsters and monkeys.

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* The chemistry of these compounds is described in Brit. Pat. 769, 706 and by J. N. Ashley, R. F. Collins and M. Davis in *J. Chem. Soc.* (in the press).

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Blood Cortisol in the Sheep: Normal Concentration and Changes in Ketosis of Pregnancy

WHEN pregnant ewes experience a sudden reduction in food intake during the last stages of pregnancy, they are prone to a disease characterized clinically by disturbances of the nervous system and by a high mortality rate[1,2]; the condition has become known as ketosis of pregnancy, pregnancy toxæmia, or twin lamb disease. Since hypoglycæmia and ketonæmia were recognized as salient features of the disease, the metabolism of carbohydrates and fatty acids in affected animals has been the subject of a large number of studies; these are discussed in recent papers[3–6]. In the past, little attention has been paid in such investigations to the functional state of endocrine glands well known to be involved in carbohydrate and lipid metabolism, such as the endocrine pancreas and the adrenal. The existing evidence concerning adrenal function in ketosis of pregnancy is indirect and conflicting. In contrast to earlier reports[1,2,5], the appearance of the gland at autopsy has recently been interpreted as indicating cortical hyperactivity[4]. On the other hand, similarities exist between ovine, ketosis of pregnancy and primary ketosis of cattle[6]; the latter condition is held to be associated with adrenocortical insufficiency, and its treatment with adrenocorticotrophic hormone or with cortisol-like drugs has been advocated[7].

Cortisol (11β : 17α : 21-trihydroxypregn-4-ene-3 : 20-dione) appears to be the principal glucocorticoid secreted by the sheep's adrenal[8]. The following summarizes results obtained by chemical determination of cortisol in the peripheral blood of ewes suffering from ketosis of pregnancy and of their normal controls. The material included field cases, as well as ewes in which the condition was experimentally induced by subjecting animals in advanced pregnancy to a fast of 3–8 days. Changes in the concentration of blood glucose and ketone bodies in these ewes were measured in the course of an independent study in this laboratory, hence diagnosis could be based on biochemical as well as on clinical findings. In 22 cases a definite diagnosis of ketosis of pregnancy appeared justified, while in 6 cases the diagnosis was equivocal.

The estimation of plasma cortisol was based on the reduction of blue tetrazolium by that fraction of the extracted neutral lipid which moved at the rate of authentic cortisol on a paper chromatogram and exhibited the yellow soda-fluorescence[9] of αβ-unsaturated 3-oxosteroids. Results were corrected for losses during analysis as indicated by the percentage of [4-^{14}C]cortisol added to plasma which could be recovered by this procedure. The limit of sensitivity of the method as routinely employed was 1·5 μgm. per 100 ml. plasma. Values for the cortisol concentration in the pooled plasma of normal human subjects (6·5 μgm. per 100 ml. mean of 8 determinations), analysed in order to test the validity of the method, were within the range of published data; further, the response to adrenocorticotrophic hormone (0·12 I.U. per kgm. body weight, by intravenous infusion) in intact sheep, and to cortisol (1·5 mgm. per kgm. administered intramuscularly) in adrenalectomized sheep, could consistently be detected.

The plasma cortisol concentration in all the samples taken from 35 normal adult Merino sheep, including animals in various stages of pregnancy, was below the range of sensitivity of this method; the concentration remained below 1·5 μgm. per 100 ml. throughout the experiment in 13 of the 14 pregnant ewes which survived a fast of 4 to 8 days without showing symptoms of the disease. On the other hand, all the 22 ewes in which a diagnosis of ketosis of pregnancy could be established showed, during the illness, a rise in plasma cortisol to readily detectable levels (4·3 ± 0·6 μgm. per 100 ml., mean ± *S.E.* of 42 samples); in the 6 moribund ewes in which the diagnosis was uncertain the cortisol concentration was measurable (1·5–3·0 μgm. per 100 ml.) in 5 of 11 samples. Results obtained when larger blood samples in healthy animals were used for analysis indicated that the normal plasma concentration in the Merino sheep is of the order of 0·5–1·0 μgm. per 100 ml. or about one tenth of that found in man. Moreover, the marked inhibition of wool growth by administration of cortisol or adrenocorticotrophic hormone, which we reported previously[10], is already apparent when the cortisol-level in the plasma of Merino sheep is maintained by such means at 2–3 μgm. per 100 ml. There is little doubt then, that the plasma cortisol concentrations found in ovine ketosis of pregnancy must be regarded as abnormally high. The 'break' in the wool fibre which frequently appears in the course of this disease may be a consequence of the attendant high blood cortisol levels, since this defect can be produced by cortisol administration in well-fed sheep[10].

We do not know whether the increased concentration of cortisol in the peripheral blood in ketosis of pregnancy is chiefly determined by an adrenal response to the severe stress imposed on the animals, or by an impaired ability of the ketonæmic ewe to metabolize and excrete the hormone. It has been shown that the rate of hydrogenation and conjugation of cortisol is much reduced in the presence of hepatic dysfunction in man[11]; the severe fatty infiltration of the liver seen in ovine ketosis of pregnancy[1,2] suggests the possibility of a similar interference. Preliminary observation showed that conjugated reduced derivatives of cortisol were still present in considerable concentration in the bile in the terminal stage of the disease, and in 2 out of 7 cases examined their concentration in the plasma was exceedingly high; however, more detailed clearance studies are required.

Of the various indices of endocrine function commonly employed, it is probably the plasma-level of the active hormone which best reflects the conditions under which the tissues have to operate. As the sheep's tissues appear to be normally adapted to very low concentrations of cortisol in their environment, it is possible that even the moderate elevation of blood cortisol seen in ketosis of pregnancy is of significance in the pathogenesis of this disease.

My thanks are due to Dr. R. L. Reid for making his experimental animals available to me, to Mr. A. M. Downes for help in the measurement of radioactivity, and to Dr. R. I. Cox for a preparation of β-glucoronidase; a generous gift of (4-^{14}C)-cortisol was received

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Effects of 1,4 Dihydrazinophthalazine on Organ Content and Adrenal Medullary Secretion of Catechol Amines

In view of possible casual relationships between the effect of reserpine on the catechol amines and its effect on the elevated arterial blood pressure in hypertension, it seemed relevant to us to study the effects on catechol amine metabolism of another type of anti-hypertensive drug, 1, 4-dihydrazinophthalazine ('Nepresoline', Ciba). The hypotensive properties of the hydrazinophthalazines were first reported in 1950 by Gross, Druey and Meier[1]. Following intravenous injection of 'Nepresoline' the blood pressure fell. This started after 10–20 min. and lasted for several hours. The hydrazinophthalazines are known to have some sympatholytic activity[2] and have been shown *in vitro* to inhibit DOPA-decarboxylase, thus possibly interfering with catechol amine synthesis[3].

In the experiments reported here, the purpose has been to study possible effects of 'Nepresoline' on the organ content of adrenaline and noradrenaline and on the secretion of medullary hormones into the adrenal vein blood.

Fifty male albino rats with an average weight of 250 gm. were divided into groups of five. The experimental groups were injected with 5 mgm./kgm. 'Nepresoline' subcutaneously twice a day during three consecutive days. The control groups received an equivalent amount of solvent. The rats were killed with a blow on the head on the first, third and fifth day after the last injection, respectively. The content of adrenaline and noradrenaline in adrenals, brain, heart and spleen was determined fluorimetrically after purification by adsorption on aluminium oxide[4]. All values were measured in μgm./kgm. body-weight. The organ content of catechol amines of the experimental groups was expressed as percentage of the corresponding values for the control groups.

The effect of intravenous injection of 'Nepresoline' (5 mgm./kgm.) on the secretion of catecholamines from the adrenal gland was studied on 8 cats, weighing 3–4 kgm. and anæsthetized with chloralose. In some of these experiments vagotomy and carotid sinus denervation was carried out. In others the left adrenal gland was denervated by acute left-sided splanchnicotomy. Test doses of 1–20 μgm. of noradrenaline standard were, in some experiments, injected intravenously before and after 'Nepresoline' to determine possible correlations between the blood pressure changes following 'Nepresoline' and changes in vascular reactivity to noradrenaline. Venous blood from the left adrenal was collected at intervals before and after 'Nepresoline', using an adrenolumbar-femoral vein shunt technique[5]. The adrenal vein plasma samples were assayed biologically on the cat's blood pressure and the hen's rectal cæcum.

In the rats killed on the first day after the injections, the organ content of noradrenaline in the 'Nepresoline' groups was significantly decreased in heart and adrenals, but not in brain and spleen, in comparison with the corresponding values for the control groups. The adrenaline values were also very low in the heart, while no significant change was found in adrenals, brain or spleen. In the rats killed on the third day all the organ values, both for adrenaline and noradrenaline, were normal. On the fifth day normal values were found, except for the adrenals, where the adrenaline and particularly the noradrenaline content was definitely lowered (Table 1).

Intravenous administration of 'Nepresoline' to cats caused a fall in blood pressure of 20–40 mm. mercury, usually after a latency of 15–20 min. This effect was closely paralleled by a decrease in the blood pressure response to exogenously administered noradrenaline. 'Nepresoline' further produced a well-marked increase in the secretion rate of noradrenaline into the adrenal vein blood, whereas adrenaline was less affected (Fig. 1). This effect appeared in the first sample 10–15 min. after 'Nepresoline' was injected

Table 1. Average organ content of adrenaline and noradrenaline in rats injected with 'Nepresoline', expressed as per cent of the corresponding values for control groups, injected with equivalent amounts of vehicle. Adrenaline values are given only for adrenals.

	Adrenaline (%)	Noradrenaline (%)			
	Adrenals	Adrenals	Heart	Spleen	Brain
1st day	115	52	17	84	81
2nd day	102	102	90	102	110
3rd day	79	36	98	117	109

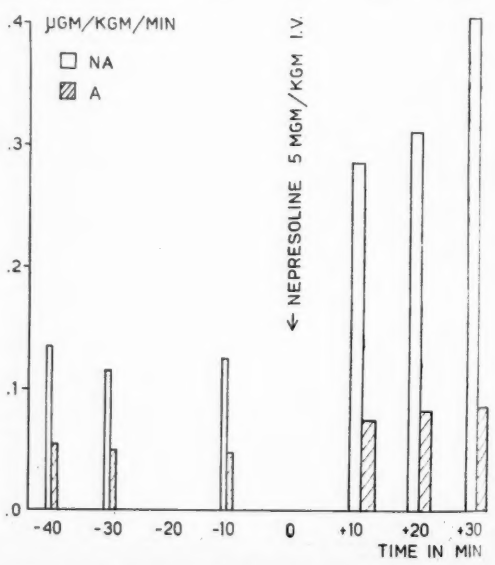

Fig. 1. Secretion of adrenaline (*A*) and noradrenaline (*NA*) from the left adrenal gland before and after 'Nepresoline', 5 mgm./kgm. (intravenous after bilateral vagotomy and carotid sinus denervation.

and was continuously increasing during the following 30–40 min. studied. The secretory response was not diminished after bilateral vagotomy and carotid sinus denervation and persisted even after left-sided splanchnicotomy.

The results of these experiments clearly indicate, that 'Nepresoline' interferes with the storage and/or release of catechol amines in the two animal species investigated.

In the rat 'Nepresoline' causes, after repeated subcutaneous injections, a partial depletion of the noradrenaline stores in adrenals and particularly in the heart, while the catecholamine content in brain and spleen are only little influenced. In the cat, a single intravenous injection of 'Nepresoline' is followed by a blood pressure fall, which is paralleled by, and possibly partly due to, its demonstrated sympatholytic activity, and by a selective increase in the noradrenaline secretion from the adrenal medulla. This secretion effect is not reflexly elicited by the blood pressure changes, caused by 'Nepresoline', since it is not diminished by vagotomy and carotid sinus denervation. This operation abolishes reflex increase in the adrenal medullary secretion secondary to a fall in blood pressure[6]. At least part of the effect is direct and independent of innervation.

It is tempting to consider the possibility that the depletion of the organ stores of catecholamines is due to inhibition of synthesis, possibly via interference with DOPA-decarboxylase. Experiments to analyse the mechanism for these 'Nepresoline' effects are in progress.

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Production of Odd-Numbered Carbon Fatty Acids from Propionate by Mice

Small amounts of fatty acids containing an odd number of carbon atoms have been isolated from the depot fat of ruminants[1–5]. The development of the gas chromatography procedure for analysis of the methyl esters of the fatty acids enabled the detection of trace amounts of the odd-numbered acids in the fats of non-ruminants[6,7]. Since fatty acids containing an odd number of carbon atoms presumably arise from the condensation of propionate and acetate derivatives[8], it would seem possible to increase these acids in the depot fats of animals by feeding propionate. This was achieved in the experiment reported herein.

Adult female mice were fed a casein-sucrose purified diet to which was added tripropionin at the expense of sucrose. The initial level of tripropionin was 10 per cent and this was increased 10 per cent each week until a 40 per cent dietary level was reached. This level was maintained for an additional 2 weeks after which the animals were killed and the depot fat isolated[9]. The fatty acid composition of the depot fats was determined by gas chromatography of the methyl esters on a column of celite-succinate polyglycol ester.

A typical chromatogram of the fatty acids from a mouse fed tripropionin is shown in Fig. 1. Peaks 3, 6 and 7 are at best barely detectable in chromatograms of the acids from the depot fat of mice fed diets not containing tripropionin. These peaks, identified from graphs of the logarithm of retention time versus chain length, represent pentadecanoic acid, heptadecanoic acid and heptadecenoic acid, respectively. Further substantiation of the identity of the odd carbon fatty acids was obtained by hydrogenation. A methanol solution (25 ml.) of the methyl esters of the fatty acids (100 mgm.) containing 5 mgm. of palladium black was agitated under an atmosphere of hydrogen until a manometer indicated that gas uptake had ceased. When the hydrogenated methyl esters were chromatographed, only 6 peaks were observed. These, identified on the basis of retention times, represent myristic, pentadecanoic, palmitic, heptadecanoic, stearic and arachidic acids. Peaks for acids containing either 13 or 19 carbon atoms were not observed.

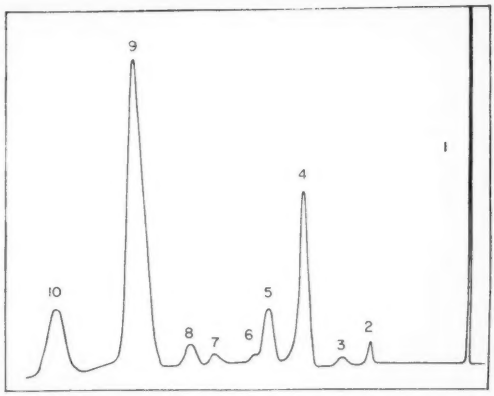

Fig. 1. Chromatogram of the methyl esters of the fatty acids from a mouse fed tripropionin. Column: succinate-polyglycol ester on 'Celite'. Temperature: 180°C. Helium flow: 50 ml./min. Peaks identified as (1) air, (2) myristic, (3) pentadecanoic, (4) palmitic, (5) palmitoleic, (6) heptadecanoic, (7) heptadecenoic, (8) stearic, (9) oleic, (10) linoleic

Table 1. Fatty acid composition of depot fat from mice fed tripropionin

Mouse No.	Saturated C_{14} %	C_{15} %	C_{16} %	C_{18} %	Monoenoic C_{16} %	C_{17} %	C_{18} %	Dienoic C_{18} %
1	1·6	1·0	17·9	2·9	7·2	1·4	55·1	12·8
2	1·5	1·2	20·1	2·8	10·3	2·1	48·7	13·3
7	2·3	1·1	20·4	3·2	11·1	1·9	46·5	13·4
Av.	1·8	1·1	19·5	3·0	9·5	1·8	50·1	13·2
Control	2·1	Tr	26·3	3·0	13·9	Tr	49·2	5·5

The fatty-acid compositions of the depot fats from the mice fed tripropionin are given in Table 1. For comparison, the average fatty-acid composition of 6 mice fed a similar diet without tripropionin is included. The amount of heptadecanoate was not included because of the poor resolution obtained. Although arachidonic acid was present in small amounts, no attempt was made to measure it because it appears as a very low broad peak on the chromatogram. In spite of the fact that large amounts of propionate were consumed by the animals, relatively low levels of odd-carbon fatty acids appeared in the depot fat. It is, therefore, apparent that the major pathway of propionate metabolism in the mouse is that of oxidation. Nevertheless, the production of odd-numbered fatty acids when propionate was fed provides further evidence that the odd-carbon fatty acids arise from the condensation of propionate and acetate units. The presence of acids containing 17 carbon atoms and the absence of acids with 19 carbon atoms indicates that a propionate unit is equivalent to the initial butyrate unit for the fatty acid synthe-

sizing system. It is also significant that the 2 principal odd-numbered carbon fatty acids found, pentadecanoic acid and heptadecenoic acid, are one carbon less than palmitic acid and oleic acid, respectively, the 2 major acids of animal fats. This is indicative that the odd-numbered fatty acids are synthesized by the same systems that produce the normal fatty acids having an even number of carbon atoms.

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Effect of Flavins on Cramp caused by Phenol

Husemann, Salkowski, Morita and others[2] have reported that phenol provokes a characteristic convulsion in animals; so the effect of flavins on cramp caused by phenol was studied.

The flavin mononucleotide was supplied by Hoffmann-La Roche Co. Flavin adenine dinucleotide was prepared by the method of Yagi *et al.*[2] The phenol used was a commercial sample. Male albino rats weighing about 130 gm. were used for the experiment.

Forty-five animals, 10 days after feeding under the same conditions were divided into three groups, each group consisting of 15 animals. The animals of the first group were injected intra-abdominally with the mixture of 1·0 ml. of 0·1 mole/l. phenol and 2·0 ml. of water; those of the second group were injected with the mixture of 1·0 ml. of 0·1 mole/l. phenol, 1·0 ml. of 0·04 mole/l. flavin mononucleotide and 1·0 ml. of water; those of the third group were first injected with 2·0 ml. of 0·02 mole/l. flavin mononucleotide, and 10 min. after the injection, 1·0 ml. of 0·1 mole/l. phenol was further injected.

Cramp was observed by electromyogram of the muscle biceps femoris. A needle electrode was placed in the muscle, and the electromyogram was recorded by a Grass model III-*D* electroencephalograph.

The time from the injection of phenol to the beginning of the convulsion (1), and that of the duration of the convulsion (2) were measured. The arithmetic mean of the results of each experiment and the mean of the population (with the probability of 99 per cent) are listed in Table 1.

The results were statistically examined, and the differences between the first group and the second or the third group were significant (with 99 per cent possibility). These results indicated that flavin mononucleotide could inhibit the provocation of phenol convulsion and shorten the duration of the convulsion. On the other hand, statistical examination of the difference between the second and the third groups showed that the difference was significant between the second group (1) and the third group (1), but not between the second group (2) and the third group (2) (with 99 per cent possibility). This result showed that flavin mononucleotide previously injected is more effective than that injected at the same time with phenol for the inhibition of the provocation of phenol convulsion, and is equally effective with that injected at the same time with phenol for the duration of the convulsion.

Table 1. Effect of Flavin on the Provocation and Duration of Convulsions caused by the Injection of Phenol

		Time from the injection of phenol to the beginning of cramp	Time of duration of cramp
First group	$\bar{X}$ ($n=15$)	1 min. 4 sec.	50 min. 6 sec.
	M ($a=0{\cdot}01$)	1 min. 15 sec.–53 sec.	53 min. 53 sec.–46 min. 19 sec.
Second group	$\bar{X}$ ($n=15$)	1 min. 17 sec.	33 min. 58 sec.
	M ($a=0{\cdot}01$)	1 min. 30 sec.–1 min. 4 sec.	37 min. 59 sec.–29 min. 57 sec.
Third group	$\bar{X}$ ($n=15$)	1 min. 34 sec.	33 min. 27 sec.
	M ($a=0{\cdot}01$)	1 min. 49 sec.–1 min. 19 sec.	36 min. 30 sec.–30 min. 24 sec.

$\bar{X}$, Arithmetic mean.
M, Mean of the population.

A similar experiment was also carried out with flavin adenine dinucleotide, and the results showed the same tendency. These results suggest that the riboflavin part of flavin nucleotides may be an active component in the above-mentioned effect.

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RADIOBIOLOGY

Assay of Plasma Insulin in Human Subjects by Immunological Methods

We have previously reported on the immuno-assay of beef insulin and certain other animal insulins, employing antiserums from human subjects treated with commercial mixtures of beef and pork insulin[1]. The insulin-binding antibodies present in these antiserums do not form precipitable complexes with insulin, but with the use of insulin labelled with iodine-131 the complexes are readily demonstrable by paper chromatography and electrophoresis[2]. Beef, pork, sheep and horse insulins can be assayed quantitatively by measurement of the degree of competitive inhibition of binding of any insulin labelled with iodine-131[1–3]. As might have been anticipated, however, human insulin competes too weakly in systems employing human antiserum to be measurable at concentrations which obtain *in vivo*. Furthermore, the lack of availability of significant quantities of pure human insulin precludes its use as an antigen for animal immunization. However, in the present work, it has been found that human insulin cross-reacts strongly with insulin-binding antibodies in guinea pigs immunized with crystalline beef insulin, and that guinea pig anti-beef insulin serum has characteristics suitable for the detection and measurement of human insulin at concentrations which exist in the plasma of normal fasting subjects.

The methods employed and results obtained in this

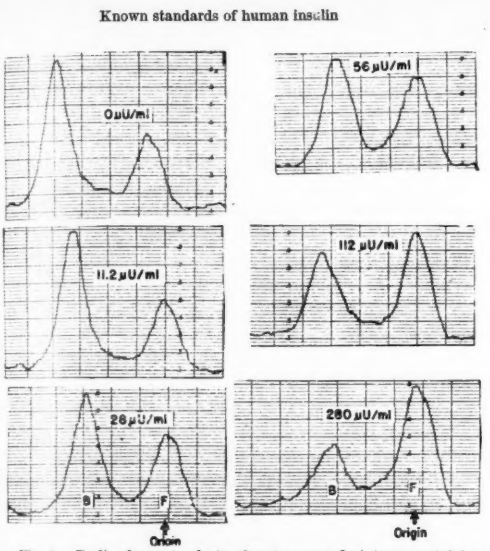

Fig. 1. Radio-chromato-electrophoretograms of mixtures containing the same concentrations of tracer crystalline beef insulin-^{131}I and guinea pig anti-beef insulin serum but varying concentrations of human insulin from known standards (left), or 1 : 4 dilutions of plasma from a human subject before and after administration of glucose (right). Two-tenths ml. of mixtures was applied to paper strips along line marked 'origin'; veronal buffer, ionic strength 0·1, pH 8·6; 15 V./cm. Free insulin (*F*) is adsorbed to paper at origin; antibody-bound insulin (*B*) migrates with serum proteins towards left under influence of water-flow chromatography and electrophoresis

work will be described in detail elsewhere. It is sufficient to note here that, following incubation of antiserum with tracer quantities of crystalline beef insulin-^{131}I of high specific activity (40–100 mc. iodine-131/mgm. insulin) radio-active insulin bound to antibody is readily distinguished from unbound ('free') insulin-^{131}I by paper chromato-electrophoresis[2] (Fig. 1) and that the ratio of bound to free insulin at trace concentrations of insulin-^{131}I can be lowered to any desired level by appropriate dilution of the anti-serum. Dilutions yielding ratios of bound to free insulin between 1 and 3 at trace insulin-^{131}I concentrations are most satisfactory for precise work. At any fixed dilution of antiserum, the ratio of bound to free insulin decreases progressively with increments in the concentration of added human insulin. The precision of the technique and the sensitivity for detection of insulin are greater according as the slope of bound to free insulin concentration is sharper. The minimum amount of tracer beef insulin-^{131}I which can be used is limited by the sensitivity of the counting system employed but must be low enough to avoid saturating the initial portion of the curve where the slope is sharpest. Best results are therefore obtained with preparations of the highest specific activity. Fig. 1 shows some representative radio-chromato-electrophoretograms. From such results a curve for bound to free insulin versus concentration of human insulin in known standards can be obtained. Table 1 gives the concentrations of human insulin in plasma samples of subjects W. and B. before and after administration of glucose.

Table 1

Subject	Endogenous insulin concentration (micro-units/ml. plasma): fasting	½ hr. after 100 gm. glucose *per os*	1 hr. after 100 gm. glucose *per os*	2 hr. after 100 gm. glucose *per os*
W	98	145	302	302
B	64	145	236	

Two to three micro-units of human insulin were detectable in the experiment depicted, but in other experiments employing beef insulin-^{131}I preparations of higher specific activity and more sensitive anti-serums, amounts of human insulin in the range 0·25–1·0 micro- units (1·25–5·0 micro-units/ml.) have been measured.

We are indebted to Drs. F. Tietze and J. Field for the human insulin employed as standard in this work.

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Behaviour of Zinc and Radiozinc in Tumour-Bearing Rats

THE distribution of zinc-65 in the tissues of rats, following intravenous injection of a '^{65}Zn-serum-protein complex', has been studied by Gilbert and Taylor[1] who showed that the zinc-65 rapidly became uniformly distributed throughout the zinc contained in the soft tissues. These authors concluded that the zinc in the soft tissues could be considered to exist in one large 'soft tissue zinc pool' freely exchanging with the zinc in the plasma. As a result of preliminary measurements in rats bearing subcutaneously implanted tumours it was suggested that the tumour could probably be considered to form part of the soft tissue pool. In this report the distribution of zinc-65 in the tissues of rats bearing a subcutaneous mammary tumour is described and it is shown that the zinc in the tumour can be considered to form part of the soft tissue zinc pool.

The animals used were male rats of the highly inbred, brown-hooded, August strain. A mammary adenocarcinoma (*R* 2426) was implanted subcutaneously into the flank of each animal 14 days prior to the intravenous injection of 0·6 μc. zinc-65 as a '^{65}Zn-serum-protein complex'. The preparation of this complex and the other experimental details have already been described[1].

The distribution of zinc-65 in the plasma, tumour and other tissues of rats killed 1–288 hr. after injection is shown in Table 1. The specific activities of the zinc-65 in the tumour, liver, kidneys and spleen of the animals killed at 96, 192 and 288 hr. after injection are presented in Table 2.

The concentration of zinc-65 in the plasma falls rapidly during the first 24 hr.; from 96 to 288 hr. the plasma concentration of zinc-65 can be described by a single exponential function:

$$P = 0{\cdot}373\ e^{-0.00011\,t}$$

where P is the percentage activity in the total plasma volume at t hr.

The half-life corresponding to this expression is 100 hr. This is much shorter than the half-life of

Table 1. Distribution of zinc-65 in tumour-bearing rats

Time	Tumour		Percentage of dose					
Hr.	Whole Tumour	%/gm.	Liver	Spleen	Kidneys	Lungs	Femur	Plasma
1	0·82	0·70	34·7	3·75	4·52	1·20	0·98	2·19
2	1·07	0·45	23·1	2·11	2·29	0·55	0·53	1·61
3	1·42	1·22	26·6	3·94	3·83	1·30	1·22	1·36
24	1·39	0·57	16·3	2·74	1·91	0·95	1·02	0·52
	1·68	0·69	14·6	3·06	1·92	0·92	1·05	0·47
96	1·04	0·306	9·1	1·71	1·08	0·73	1·13	0·17
	1·63	0·317	9·8	0·80	1·00	0·67	1·51	0·22
192	1·33	0·185	4·55	—	0·52	0·42	1·37	0·11
	1·56	0·120	5·73	0·44	0·52	0·43	1·11	0·12
288	1·53	0·089	5·59	0·14	0·47	0·26	1·30	0·051
	0·85	0·100	3·91	0·15	0·45	0·23	1·22	0·064

Table 2. Specific activity of zinc-65 in tissues of tumour-bearing rats

Tissue	% injected zinc-65 per μgm. zinc at time :					
	4 days		8 days		12 days	
Tumour	0·044	0·034	0·016	0·018	0·016	0·015
Liver	0·040	0·036	0·020	0·017	0·015	0·015
Spleen	0·042	0·040	—	0·019	0·017	0·018
Kidneys	0·036	0·034	0·020	0·020	0·016	0·020

203 hr. found for normal rats of the same strain and age, measured over the same period[1].

The specific activity data given in Table 2 show that from 96 hr. onward the specific activity of the zinc-65 in the tumour, liver, kidneys and spleen is identical. This suggests that the zinc in the tumour behaves in the same way as the zinc in the other soft tissues, and that it is freely exchangeable with the plasma zinc. The zinc contained in the tumour can therefore be considered to form part of the soft tissue zinc pool in these animals.

The observations reported here refer to the specific activity of the total zinc in the tumour and other tissues and, while the results suggest that the overall behaviour of the zinc in the tumour is similar to that in the other tissues, it is possible that the intracellular distribution of zinc may be markedly altered in the tumour cells. Bartholomew *et al.*[2] have shown that the distribution of zinc within the cells of a mouse mammary carcinoma is markedly different from that in the cells of normal mouse mammary tissue.

Limited studies using zinc-65 in two human subjects suffering from cancer, suggest that the behaviour of zinc in the human in essentially the same as in the rat, and that the zinc in human soft tissues, including tumour and metastases, can also be considered to exist in one large soft tissue zinc pool.

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Preliminary Observations on the Fate of Intravenously Administered Lactose Labelled with Carbon-14

It has been generally accepted that lactose when injected into the blood stream is not metabolized, but is excreted unchanged (see, for example, the review on lactose by Herzenberg[1]). However, results[2] obtained in this laboratory using lactose labelled with carbon-14 indicate that when administered intravenously it is not excreted unchanged but is, to a lesser degree, metabolized and also excreted, not only as lactose, but also in other forms.

Several male adult Sprague-Dawley rats received caudal vein doses of 100 mgm. each of either chromatographically pure lactose-1-^{14}C (glucose moiety labelled in the 1 position) or lactose in which all the carbons were labelled[3] (lactose-*U*-^{14}C) The animals were kept on test for 24 hr. Expired air, urine, and fæces were collected during this period after which the animals were killed. Based on the radio-activity injected into the animals, quantitative yields were obtained on the recovery of carbon-14 radio-activity. In the experiments in which lactose-1-^{14}C or lactose-*U*-^{14}C were used, an average of 5 per cent of the administered radio-activity was found in the expired air while approximately 80 per cent of the radio-activity appeared in the urine. The balance of the activity was found in the carcass.

The carbohydrate fraction in the urine was paper chromatographed and scanned[4]. A scanning record of both carbohydrate fractions is shown in Figs. 1 and 2. With lactose-1-^{14}C (Fig. 1), the majority of the radio-activity appearing in the urine was represented by lactose with at least three other components, assumed to be oligosaccharides, contributing a small fraction of the total radio-activity. With lactose-*U*-^{14}C (Fig. 2), the radio-activity was found to reside equally in at least six components of which one was lactose.

These differences in the radio-active patterns can be explained if it is assumed that a β-galactosidase type hydrolysis of lactose has occurred. The initial reaction[5] of the enzyme on lactose is the formation of a galactosido-enzyme complex with the liberation of the second monosaccharide residue, glucose. The formation of a galactosyl oligosaccharides is the result of a transgalactosidation reaction in which galactose in the form of a galactosido-enzyme complex reacts as a donor molecule with various acceptors.

The scanning record obtained with lactose-1-^{14}C (Fig. 1) coincides with this explanation. Since the galactose derived from lactose-1-^{14}C is unlabelled, the synthesized oligosaccharides would be radio-active

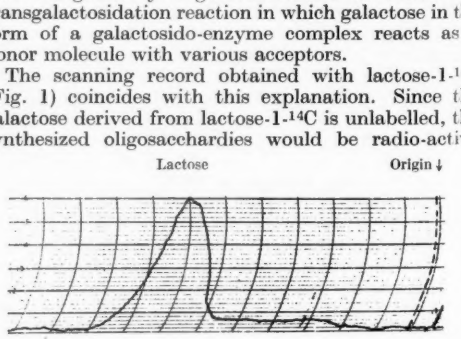

Fig. 1. Scan of urine sugar fraction from caudal vein injection of lactose-1-^{14}C. Scanner sensitivity, 1,000 c.p.m.; slit width, 2·0 mm.

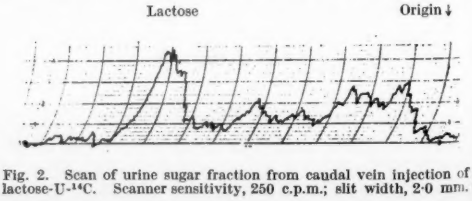

Fig. 2. Scan of urine sugar fraction from caudal vein injection of lactose-U-^{14}C. Scanner sensitivity, 250 c.p.m.; slit width, 2·0 mm.

only when the acceptors were glucose-1-^{14}C in the form of the free hexose or as a monosaccharide moiety of an oligosaccharide. With lactose-*U*-^{14}C, the donor molecule, galactose, is labelled resulting in a marked increase in oligosaccharide radio-activity (Fig. 2).

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HÆMATOLOGY

Methæmoglobin Reduction through Cytochrome b_5

HUENNEKENS *et al.*[1] purified an erythrocyte fraction containing hæm which was able to catalyse the reduction of methæmoglobin by reduced triphosphopyridine nucleotide in the presence of methylene blue. A single enzyme was believed to be responsible for the electron transport from reduced triphosphopyridine nucleotide to the dye, and from the dye through hæm to methæmoglobin. Although reduced diphosphopyridine nucleotide-cytochrome *c* reductase from pig heart and reduced triphosphopyridine nucleotide-cytochrome *c* reductase from yeast do not reduce methæmoglobin, it was shown by Cromier and Rostorfer[2] that bacterial extracts with diaphorase activity, as well as milk xanthine oxidase, do reduce methæmoglobin in the presence of methylene blue, suggesting that diaphorases could also act similarly to methæmoglobin.

One of us previously isolated from pig liver[3] (H. R. Mahler, and I. Raw, unpublished work) also a cytochrome, identical to cytochrome b_5. Rapid purification of the cytochrome was achieved by treating the ammonium sulphate fraction, obtained in a manner previously reported[3], with 'DEAE'-cellulose at *p*H 6 in 0·001 *M* potassium phosphate buffer, to adsorb most of the cytochrome, leaving behind the hæmoglobin. The cytochrome was then eluted with *M*/4 potassium phosphate buffer, *p*H 7 and further purification was obtained by chromatography in hydroxyapatite[3], and irradiation at 0° C., for 3 hr. with short-wave ultraviolet light ('Mineralight' model *V*–41) to destroy residual reductase.

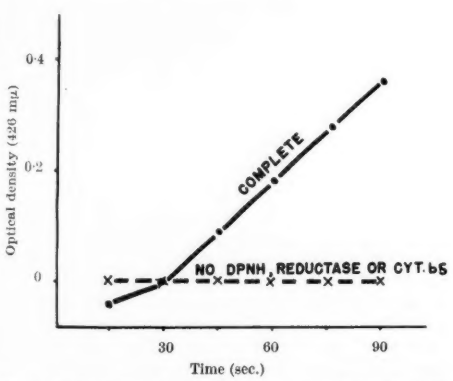

Fig. 1. Reduction of methæmoglobin by diphosphopyridine nucleotide. The system contained in 2·3 ml. $1{\cdot}4 \times 10^{-4}$ moles of methæmoglobin (prepared or ferricyanide reduction of crystaline horse hæmoglobin) 0·5 γ of diphosphopyridine nucleotide-cytochrome b_5 reductase $SA = 60$, $1{\cdot}4 \times 10^{-6}$ moles cytochrome b_5 (or 10^4 moles methylene blue) and 2×10^{-2} moles 426, and $\Delta\varepsilon$ assumed to be 57×10^6 (ref. 5). Methæmoglobin was added at 0 time after pre-incubation of the other components for 2 min., at 25° C.

A specimen of reduced diphosphopyridine nucleotide-cytochrome b_5 reductase from pig liver was used and prepared as previously described[4]. This enzyme did not reduce methæmoglobin directly.

Fig. 1 shows that methæmoglobin is reduced by reduced diphosphopyridine nucleotide in the presence of the reductase and cytochrome b_5. As expected, methylene blue cannot be substituted for the cytochrome. The rate of reaction is, within experimental error, equal to the rate of cytochrome b_5 reduction, and linear after the first 30 sec.

Present results agree with Huennekens *et al.*[1] that methæmoglobin can be reduced by a reduced hæmoprotein. It is interesting to note that the best preparation of erythrocyte methæmoglobin reductase reduced about 0·03 μmole/min./mgm. of protein, whereas a rate of 200 μmole/min./mgm. of protein is obtained with purified reduced diphosphopyridine nucleotide cytochrome b_5 reductase from pig liver. This would suggest that the erythrocyte methæmoglobin reductase may be a similar multi-enzymatic system, but lacking one fully active component.

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Banding of Hæm to Protein in Hæmoglobin and Myoglobin

RECENTLY it was postulated that in hæmoglobin and myoglobin the hæm iron is bound to the apoprotein other than through histidine imidazole groups[1]. This conclusion, which does not appear to be warranted by evidence at present available, is based on the assumption that the *p*H at which half the hæm is bound to the protein moiety can be identified with the pK_L of the binding group. There is no theoretical reason why this should be so.

Assuming the binding between the hæm and the protein to involve co-ordination between the iron atom and a ligand group on the protein, then *p*H (50 per cent) is related to pK_L, to the stability constant (K_s) of the complex and to the total ligand ($[L]_T$) and metal ($[M]_T$) concentrations, in the following manner :

$$p\text{H (50 per cent)} = pK_L + pK_s - \log_{10}(L_T - \tfrac{1}{2}\,M_T - {}^1/_{K_s})$$

Clearly, pH (50 per cent) cannot equal pK_L except in very special circumstances. If other interactions of, for example, Van der Waals or electrostatic nature are involved in the binding, the relationship of pH (50 per cent) to pK_L becomes even more complicated. Thus measurements of this kind cannot lead to values of pK_L unless the other factors are known.

To interpret potentiometric studies of horse carboxyhæmoglobin[2], seven different pK values were necessary, representing acid groups, histidine groups and lysine, tyrosine or arginine groups, but the authors did not suggest which of these groups were linked to hæm. Wyman[3] has presented evidence that the bonding groups in horse hæmoglobin are imidazole portions of histidine as suggested originally by Küster[4] and more explicitly by Conant[5]. Carboxyl and sulphydryl groups also have been suggested as the bonding group from globin, and some evidence for and against these, and histidine, has been summarized by Haurowitz[6]. For reasons discussed above, O'Hagan's results throw no light on the nature of the bonding group.

If there are changes in the degree of aggregation of any of the reacting species, it is even less justifiable to identify pH (50 per cent) with pK_L. It is true that pH (50 per cent) will equal pK_L if the experimentally unrealistic assumption is made that complex formation between hæm and globin is so slight that the hæm-globin complex has a stability constant approaching zero; in other words, that the hæm is not bound to the protein.

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The equation deduced by Drs. Falk, Phillips and Perrin would seem applicable only when interaction between the 'metal' and ligand would be high, for example, in the hæm compounds which have reacted with oxygen or carbon monoxide. If the interaction were low, as it seems to be in the hæmatin compounds on which my experiments were performed, where dissociations appear to be simple (for example, in hydroxide formation) and the linkages 'essentially ionic', then the second and third terms on the right-hand side of the equation could be discarded. My interpretation assumed this, and that removal of the insoluble hæmatin, with polymerization and loss of absorbance, 'indicated' the dissociation of the bonding group of the protein. It was also assumed that over the range of hydrogen ion concentration studied, contributions to the absorbance from linkages to the hæmatin propionate side-chains would be low. The latter opinion now appears confirmed, since the dissociation of ferriætiomyoglobin (prepared from ætiohæmin, with no carboxyl groups) apparently follows the theoretical curve for an acid group with $pK = 4{\cdot}95$ (at 21°, $\mu = 0{\cdot}05$)[1].

In discussing the potentiometric studies on horse carboxyhæmoglobin, Cohn, Green and Blanchard[2] listed four carboxyl groups under a Kp'_3 value of 4·8. They stated: 'The total curve fits better if a small number of groups—possibly representing four of the groups of hæmatin—is assumed to dissociate in the neighbourhood of pH 4·9'. This statement is perhaps ambiguous, but the 'small number of groups' apparently refers to the groups of the protein attached to the four hæmatins, rather than to four of the eight carboxyl groups of the hæmatins, since these would be expected to dissociate with $pK \sim 5{\cdot}7$ (ref. 3). Haurowitz[4], on completely different evidence, does not consider the hæm iron to be bound to imidazole residues, but that hydroxyl or carboxyl group binding is more likely. A group of more negative character than imidazole has previously been considered in the case of myoglobin[5], and it has perhaps been forgotten that German and Wyman[6] considered the groups might be 'either imidazole groups of histidine, or the second carboxyl groups of dicarboxylic acids'.

It has been shown elsewhere[1] that rather than those imidazole side-chains of horse oxyhæmoglobin ionizing at neutrality being co-ordinated with the iron atom, their electrostatic linkage to the propionate side-chains of the oxygenated hæms is more likely. Consideration of the curve for the apparent heat of dissociation of horse oxyhæmoglobin[7] shows that in the region about pH 5, where more than half the ligand groups of the protein are still linked to the hæm iron, the groups dissociating have $\Delta H_{app.} \sim 0$, which is very suggestive of carboxyle groups. The hæmoglobin shows little loss of capacity to combine reversibly with oxygen down to pH 4·5 (ref. 8), so that linkage of the hæm iron to carboxyl rather than imidazole groups is indicated.

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Multiple Hæmoglobins in Fish

Though there have been reports by different workers[1,2,3] about the concentration of hæmoglobin in different species of fish, no information seems to be available in the literature about hæmoglobin patterns of fish. This communication deals with the findings on the blood samples of 10 different species of freshwater fish: *Catla catla*, *Labeo rohito*, *Cirrhina mrigala*, *Labeo calbasu*, and *Labeo bata* (family Cyprinidæ), *Ophicephalus punctatus* Bloch and *Ophicephalus striatus* Bloch (family Ophicephalidæ), *Heteropneustes fossilis* (Bloch) (family Heteropneustidæ), *Clarias batrachus* (Linn) (family Claridæ) and *Tilapia mosambica* (family Cichlidæ). After this note had been prepared, a report[4] on the occurrence of multiple hæmoglobins in some species of fish has appeared.

Blood samples were obtained direct from the heart

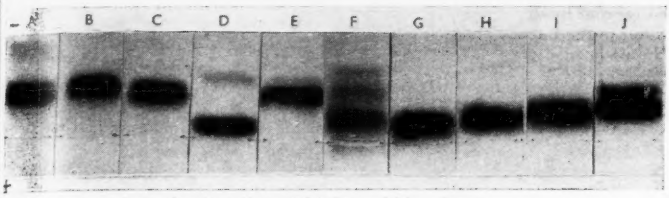

Fig. 1. Agar-electrophoretic pattern of fish hæmoglobins. Arrow indicates point of application. *A*, *C. catla*; *B*, *L. rohito*; *C*, *C. mrigala*; *D*, *L. calbasu*; *E*, *L. bata*; *F*, *O. punctatus*; *G*, *O. striatus*; *H*, *H. fossilis*; *I*, *C. batrachus* and *J*, *T. mosambica*

after dissection of the live fish, into clean heparinized tubes. Blood hæmolysates were prepared and the analysis carried out by the agar electrophoretic technique[5,6]. The hæmoglobin bands appeared blue on staining with amido-Schwarz. The bands, immediately after electrophoresis, were tested with the benzidine reagent and were confirmed as hæmoglobins. The samples which showed the presence of more than one hæmoglobin were analysed by use of a photovolt densitometer (Photovolt Corporation model 52-*C*) to evaluate the percentage distribution of the different components. Fig. 1 presents photographs of typical electrophoretic patterns of the fish blood samples. Table 1 gives the number of hæmoglobins and the percentage distribution of the different hæmoglobins in each of the ten species of fresh-water fish investigated.

Table 1 shows that the blood of *O. punctatus* contains three hæmoglobins, *C. catla*, *L. calbasu*, *L. bata* and *T. mosambica* two, and the other five species only one each. All the five species having multiple hæmoglobins have a major component (α) and one or more minor components (β, γ). The hæmoglobin components are designated as α, β and γ in the order of decreasing electrophoretic mobility. The α-components of all the species account for nearly 70–90 per cent of the total hæmoglobins, β and γ representing only a much smaller fraction. Even in the electrophoretic mobility, the different species exhibit appreciable differences. The major components of *C. catla*, *L. rohito*, *C. mrigala* and *L. bata* have nearly the same mobility, whereas that of *L. calbasu*, *O. punctatus*, *O. striatus*, *C. batrachus*, *H. fossilis* and *T. mosambica* have a slightly higher mobility. All the components of fish hæmoglobins have a low mobility similar to those of γ-globulins of human serum or even lower.

The blood samples investigated are all from adult fish, and all members of the same species invariably showed a consistently similar hæmoglobin pattern. This observation is of significance in the light of reports that tadpole and adult frog show slightly different hæmoglobin patterns[7] and that some species like sheep and cattle show a mixed pattern, some members having two hæmoglobin components and others having only one[8,9,10]. The hæmoglobin patterns in fish do not seem to conform to any distinct family characteristic. Even within the air-breathing species there is significant heterogeneity in their hæmoglobin patterns.

Table 1. HÆMOGLOBINS OF FRESH-WATER FISH

Species	No. of hæmoglobins	Percentage (of total) composition of hæmoglobin components		
Catla catla	2	87·72 ±6·81	12·29 ±6·81	—
Labeo rohito	1	—	—	—
Cirrhina mrigala	1	—	—	—
Labeo calbasu	2	90·18 ±3·20	9·83 ±3·20	—
Labeo bata	2	92·88 ±0·61	7·12 ±0·61	—
Ophicephalus punctatus	3	70·67 ±5·64	22·69 ±4·12	6·63 ±2·59
Ophicephalus striatus	1	—	—	—
Heteropneustes fossilis	1	—	—	—
Clarias batrachus	1	—	—	—
Tilapia mosambica	2	91·16 ±1·84	8·84 ±1·84	—

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Paper Electrophoresis of Hippopotamus Hæmoglobin

THE study of animal hæmoglobin types is developing rapidly and the results obtained in various species have recently been summarized by Huisman, van der Helm, Visser and van Vliet[1]. However, the electrophoretic mobility of hippopotamus hæmoglobin does not seem to have been studied before, possibly because of scarcity of materials. We have had the opportunity to examine hæmoglobin samples from the East African hippopotamus, *Hippopotamus amphibius* L.[2], obtained during a controlled reduction in a hippopotamus population.

Samples were obtained from forty adult hippopotami of both sexes and after extraction they were examined by paper electrophoresis on Whatman No. 100 strips at *p*H 8·6 in Veronal buffer of ionic strength 0·05. A current of 1·5 m.amp. per strip was applied for six hours. For reference, comparisons were made with a sample of bovine blood (*Bos indicus*), known to contain[3] both the hæmoglobins bov. A and B. To complete the study, hæmoglobin samples were also obtained from European domestic pigs of the Large White breed from a herd established at Entebbe[4] and also from two tame specimens of the East African bush pig, *Potamochœrus porcus korinopotamus*[2].

Our investigations showed that a single hæmoglobin band was present in all the samples examined. The hippopotamus hæmoglobin migrated at a speed comparable to the slow-moving cattle hæmoglobin, bov. A.

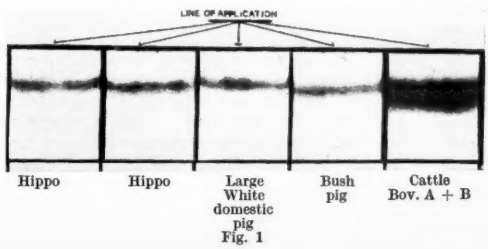

Fig. 1

The reference samples from Large White Pigs and bush pigs also showed a single hæmoglobin which moved at a similar speed in these conditions. Representative specimens are shown in Fig. 1.

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HISTOLOGY

Renewal Times of some Squamous Epithelia in the Guinea Pig

The lesions of foot-and-mouth disease in the guinea pig are localized in the epithelium of the mouth, feet, prepuce, ear and sometimes in other hairless parts of the skin[1]. During an investigation[2] into the factors responsible for this, information was sought on the quantitative aspects of epithelial growth at the predilection sites as compared with hairy skin. As data on this subject are scanty in the guinea pig[3], some investigations were carried out. In the present communication, values for the maximum renewal times and other data are reported for the uncornified layers of the epithelium of hairy skin, ear, metatarsal pad, prepuce and tongue. The differing characteristics of these epithelia are shown in Fig. 1.

Twenty male albino guinea pigs, 600–690 gm. from the Institute colony were housed in bins in a room at 21° (19–23°) and fed on bran mash and green food. The time of the year was early April. Renewal times were determined by a modification of Storey and Leblond's method[4]. Groups of five animals were injected subcutaneously with 0·65 mgm. of colchicine at 10 a.m., 4 p.m., 10 p.m. and 4 a.m. (G.M.T.) respectively and killed 6 hr. after injection. Tissues were fixed in 10 per cent neutral buffered formalin after gentle extension where necessary. Paraffin sections 7μ thick were stained with hæmatoxylin and eosin. The sites investigated were the epidermis and follicle mouths (but not the follicle necks) of the hairy skin of the flank, the distal third of the outer surface of the ear, the central region of the metatarsal pad, the epithelium of the preputial orifice and the tongue dorsum immediately behind the tip.

To minimize errors arising from the uneven distribution of mitoses, the following procedure was used for determining the mitotic index. A length of each epithelium was demarcated with Indian ink on the coverslip and projected at ×25 magnification. The surface length was determined with a map measurer and a microscopical count of all epithelial cell mitoses within the marked boundaries was made. The total numbers of nuclei in 5 or more full-thickness sectors of epithelium, each 100μ long in terms of epithelial surface, were then counted in projected images (×450).

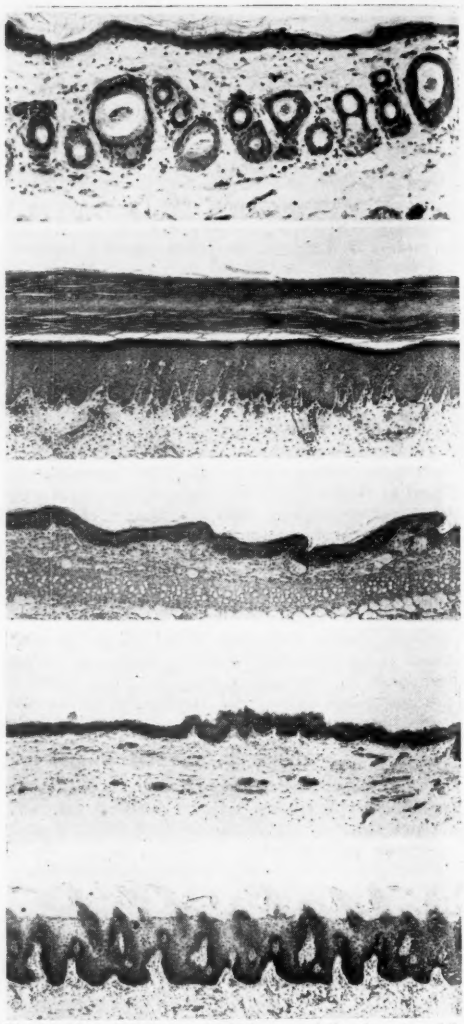

Fig. 1. Marked differences in thickness and the character of the basal layer. Top to bottom: hairy skin; metatarsal pad; skin of ear; prepuce; tongue dorsum. Hæmatoxylin and eosin (×36)

Table 1. Renewal Times of Squamous Epithelia in the Guinea Pig

Period	Percentages of cells in mitosis (Mean and S.D.)				
	Hairy skin	Ear	Metatarsal pad	Prepuce	Tongue
10 a.m.– 4 p.m.	0·35±0·12	0·29±0·15	0·44±0·25	1·25±0·55	4·54±2·17
4 p.m.–10 p.m.	0·30±0·05	0·16±0·08	0·35±0·21	0·70±0·17	2·44±0·47
10 p.m.– 4 a.m.	0·33±0·15	0·13±0·03	0·18±0·20	0·84±0·53	2·13±0·67
4 a.m.–10 a.m.	0·24±0·18	0·12±0·06	0·21±0·04	0·83±0·27	2·82±0·15
Percentage of cells entering mitosis/24 hr.	1·22	0·70	1·18	3·62	11·93
Renewal time (days)	82·0	142·9	84·7	27·6	8·4

Table 2. Mitotic Activity and Extent of Basal Layer in Squamous Epithelia

	Hairy skin	Ear	Metatarsal pad	Prepuce	Tongue
Mitoses/mm. of basal layer (Mean and S.D.)	1·73±0·66	1·72±1·26	2·31±1·21	*8·80±10·60	24·82±6·19
Ratio of length of basal layer: surface of Malpighian layer	0·97±0·05	1·22±0·15	2·76±0·30	1·06±0·09	2·69±0·28

* When one discordant observation is excluded a value of 4·06±0·78 is obtained.

The mean value together with the known length of the epithelium permitted an estimate of the total epithelial cell population in the demarcated zone. The percentage incidence of mitosis for each 6-hr. period was corrected to allow for slight differences in the diameters of resting and mitotic nuclei[5]. The renewal time for each epithelium was then calculated from the percentage of cells entering mitosis per day. The results are shown in Table 1.

Skin, whether of metatarsal pad, ear or flank, had a longer renewal time than the mucosal-type epithelia of the prepuce and tongue. The renewal time of the metatarsal pad was longer than that found by Volkmann[6]; but in his experiments, local trauma may have accelerated the rate of renewal. The short renewal time for the tongue is similar to that reported by Henry *et al.*[7] for the oral epithelium of the rabbit.

Differences were found in the incidence of mitosis at different times of the day. In each type of epithelium, the highest values were in the period 10 a.m.–4 p.m., while the lowest occurred at times varying with the epithelium. The differences between the highest and lowest values were significant ($P<0.05$) in the case of the tongue and the ear. This would suggest that mitotic periodicity occurs at least in some of these epithelia, and that, as in the mouse[8], the peak mitotic activity is during the daytime.

Since cellular replacement in squamous epithelia depends on mitotic activity in or near the basal layer, attention was given to the incidence of mitoses per millimetre of basal layer, and the extent of the latter in relation to the surface of the granular layer. For this, additional sections from the material in the 10 a.m.–4 p.m. group were measured at $\times 200$ magnification. In the tongue, measurements of the granular layer surface included the contours of the cores of the filiform papillæ (Table 2).

The number of mitoses per millimetre basal layer did not differ significantly between hairy skin, ear and metatarsal pad, and may represent an optimum inherent in cutaneous epithelium. In the thick metatarsal pad epithelium, the requisite supply of new cells is maintained by the large extent of the basal layer rather than by any undue mitotic activity per unit length of it. On the other hand, the rapid renewal of tongue epithelium is achieved by the high mitotic activity of the basal layers coupled with a large basal layer/surface ratio.

The extent to which such differences are determined by properties inherent in the epithelia themselves as compared with local irritative and temperature factors is not clear. However, it has been shown that the mitotic activity of metatarsal pad increases when grafted to the chest, while that of tongue epithelium declines[2]. It would seem, therefore, that local environmental factors must indeed play some part.

It is unlikely that the renewal times or the mitotic activity of the different epithelia are determining factors in the localisation of lesions in foot-and-mouth disease.

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VIROLOGY

Purification of Herpes Simplex Virus by Chromatography of Calcium Phosphate

The chemical and immunological analysis of herpes simplex virus cannot be undertaken until the virus has been purified without being disrupted or denatured. The purification of virus suspensions of strain *HFEM* grown in eggs[1] or in HeLa cell cultures[2] was therefore attempted by the method of Taverne, Marshall and Fulton[3] in which virus suspensions are passed down columns of calcium phosphate at room temperature and the adsorbed material then eluted with increasing concentrations of phosphate buffer at *p*H 7·0. The technique used here differed in two minor details. First, before chromatography, phosphate buffer was added to the virus suspensions to a final concentration of 0·2 *M*; this reduced adsorption of protein but not of virus. Secondly, the eluting buffers usually contained 0·1 per cent of bovine serum albumin (Armour fraction V) to reduce loss of virus infectivity. (Albumin did not adsorb in phosphate concentrations greater than 0·2 *M*.) Infective virus was titrated by pock counting, and it was shown that the infectivity titre did not drop when the virus was held in 1·0 *M* phosphate for 15 min. at room temperature. Complement-fixing activity was measured by the method of Fulton and Dumbell[4] using overnight fixation at 4° C. and human immune serum. Protein was estimated colorimetrically[5].

Infected amniotic fluid. Fig. 1 shows the results of a representative experiment with virus grown in eggs. Infectivity, complement-fixing activity and protein were separately estimated. In this experiment bovine albumin was not included in the eluting buffers.

Infective virus was largely contained in two fractions (0·4 *M* and 0·7–0·8 *M* phosphate), the latter fraction almost always being opalescent. When the two fractions were rechromatographed after dialysis or dilution with distilled water, each was again eluted by the concentration of phosphate which had eluted it from the first column. At least 50 per cent of the initial number of pock-forming units (pock U) was always recovered. About 1 per cent of the initial pock U never adsorbed (see filtrate fraction on abscissa); this was not due to saturation of the column.

Complement-fixing activity was greatest in amount in the 0·2 *M* fraction. The ratio complement-fixing activity/pock U, however, was highest in the filtrate (which also contained the small, but constant, fraction of virus which did not adsorb). The separation of infective virus from most of the complement-fixing activity by chromatography supports the finding of Hayward[6] and others which suggests that herpes complement-fixing activity detected by human immune serum is chiefly due to a smaller component than the virus particle itself.

Protein was largely contained in the 0·3 *M* and lower fractions. The ratio: pock U/mgm. protein was ten times higher in the two peaks than in the crude virus suspension, while the concentration of pock *v*/ml. was at least 30 per cent of the initial figure, which was usually $1–2 \times 10^8$ pock U/ml. Thus chromatography on calcium phosphate permits substantial purification without much loss of infectivity.

HeLa cell virus. Fig. 2 shows the results of chromatography of virus grown in cultures of HeLa

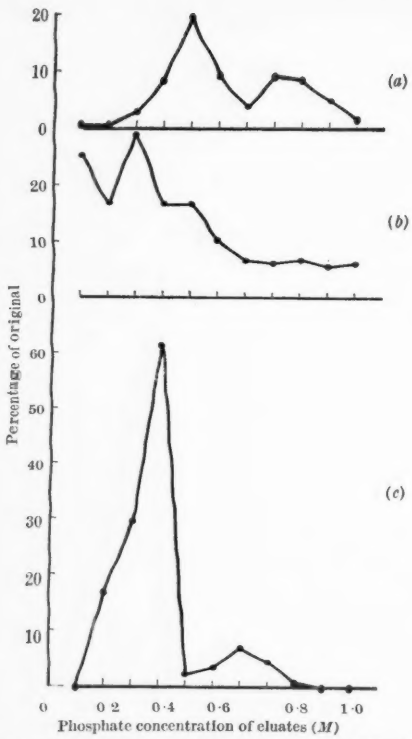

Fig. 1. Chromatography of herpes-infected amniotic fluid: the distribution of infective virus, complement-fixing activity and protein in the eluates

a, Pock *U*; *b*, complement-fixing activity; *c*, protein

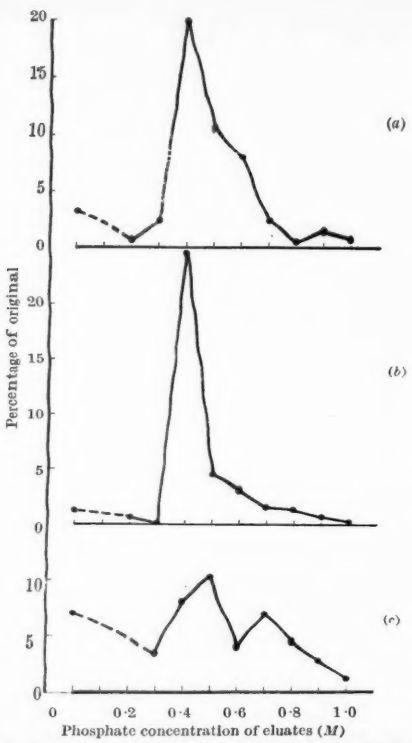

Fig. 2. Chromatography of HeLa cell virus from (*a*) infected tissue culture fluids. (*b*) versene washings of infected HeLa cells and (*c*) a suspension of disrupted, versene-washed HeLa cells: distribution of infective virus in the eluates. Note.—Phosphate was added to (*a*) and (*b*) to a final concentration of 0·2 *M* and to (*c*) to 0·3 *M*, before chromatography

a, Supernatant fluid; *b*, rescue washing; *c*, disrupting cell extract

cells. Most of the virus from infected tissue culture fluids was eluted in one fraction, at 0·4 *M* phosphate. Virus obtained by washing infected HeLa cells with 1/5,000 versene (sodium ethylene diamine tetra-acetate) also eluted mainly in one fraction at 0·4 *M* phosphate. In contrast, virus released by disrupting versene-washed cells in a micro-blender eluted in two major fractions, one at 0·4–0·5 *M* and one at 0·7 *M*. It seems possible, therefore, that cell-associated virus may have a different physico-chemical character from that released either spontaneously by the cell or obtained by washing the cell surface with versene. It is not known whether the second, 0·7 *M*, fraction observed with egg-brown virus represents similar cell-associated virus. It is of interest, however, that virus particles embedded in cell debris were seen in electron micrographs of disrupted infected HeLa cells[7].

It is well known that virus suspensions are very heterogeneous. Several other viruses have been reported to contain more than one infective component separable by physico-chemical methods. For example, Type 1 poliomyelitis virus[8] and Rous sarcoma virus[9] have both been shown by zone electrophoresis to contain at least two infective components having different mobilities. The *PR*8 strain of influenza *A* virus has been shown by chromatography on an anion exchange resin to contain at least three infective components indistinguishable by complement fixation or serum neutralization[10]. The significance of the two, possibly three, components of herpes virus is not yet clear. It is not known whether they are serologically or genetically different. Further work on their properties is in progress and will be reported elsewhere.

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Serum Inhibitors of Asaian Strains of Influenza Virus

During the past several months we have been studying the serological behaviour of various Asian influenza strains, with particular reference to their sensitivity to non-specific serum hæmagglutination inhibition (HI). Of the five strains studied, four were highly sensitive, one moderately so, to human serum

inhibitor in early egg passages. Sensitivity was lost abruptly on continued passage in the allantoic cavity in four of the strains. More detailed studies on one of the strains, *A*/Roberge, have shown that loss of sensitivity to inhibition of hæmagglutination is correlated with (*a*) resistance in *ovo* to the neutralizing activity of normal horse serum and (*b*) increased enzymatic activity as measured by rapidity of elution from chicken red blood cells.

Agglutination of 0·5 per cent chick red blood cells by 8 agglutinating doses (AD) of *A*/Roberge was inhibited (during the first six egg passages) by high dilutions (1/640 to 1/2560) of 14 out of 44 normal human sera tested. Significant inhibitory activity was also shown by normal guinea pig and rabbit sera; normal mouse serum was ineffective. Heating tended to enhance, rather than destroy the serum inhibitor. All inhibitory activity was destroyed by treating sera with *M*/90 potassium periodate at room temperature. Trypsinization lowered but did not completely remove inhibitor titres.

On the seventh and subsequent passages in eggs, *A*/Roberge was completely resistant to inhibitor in all sera tested; no inhibition was observed at serum dilutions of 1/10.

Cohen and Belyavin[1] recently reported than an inhibitor distinct from the known (alpha- and beta-) components, and present in high titre in normal horse serum, was active against Asian strains; the inhibitor possessed considerable neutralizing activity *in ovo*. Tests performed with *A*/Roberge showed that it was extremly sensitive to the normal serum component (complete inhibition by serum dilution above 1/5000) up to the sixth egg passage; no inhibition was present when the seventh and later passages were tested. The same pattern of sensitivity and resistance was shown in neutralization tests (Table 1). When fourth passage (*E*4) virus was inoculated in a medium containing 10 per cent horse serum a drop of nearly 4 logs in the infectivity titre was observed. *E*7, resistant to hæmagglutination inhibition by horse serum, showed no drop in infectivity.

Table 1. Neutralization Tests with Sensitive and Resistant Virus of the *A*/Roberge Strain

No. of egg passage	Horse serum HI titre	Control *EID*50	*EID*50 when passed with 10 per cent horse serum
4	> 5,000	$10^{5.3}$	$10^{1.8}$
7	< 10	$10^{5.6}$	$10^{5.7}$

Virus recovered from passage of *E*4 with horse serum (*E*5–*hs*) was resistant to hæmagglutination inhibition by horse serum as compared to normal *E*5, which was sensitive to serum dilutions of 1/5000 or greater. Thus, the selective influence imposed by horse serum inhibitor resulted in recovery of resistant virus earlier than usual in the egg passage series. It is probable that the same sort of selection occurs naturally during allantoic passage. Preliminary experiments indicate that a component with hæmagglutination inhibition activity against the *A*/Roberge strain is present in low concentration in normal allantoic fluid.

Table 2. Elution of Virus from Chicken Red Blood Cells

No. of egg passage	Horse serum HI titre	Percentage elution–time after complete adsorption: 0 min.	30 min.	60 min.	3 hr.
4	> 5,000	0	6·6	6·6	39·8
7	< 10	0	48·3	48·3	75·0
5	> 5,000	0	1·6	6·3	25·5
5-hs	< 10	0	25·8	25·8	51·6

The possibility that increased enzymatic activity was responsible for resistance to serum inhibitor was tested by adsorption–elution experiments, using a modification of Hirst's[2] procedure. The following comparisons were made: (*a*) early passage virus late passage virus and (*b*) *E*5 *E*5–*hs*. (Table 2.)

In both samples of resistant virus—namely virus recovered by extended allantoic passage and that recovered by using the selective influence of horse serum inhibitor—elution is more rapid and more complete over the period of observation than for the sensitive virus.

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Appearance of Crystalline Needles in Monkey Kidney Cell Cultures Infected with Poliomyelitis Virus and Incubated at 22°–30° C.

A cytochemical study of the distribution of ribonucleic and deoxyribonucleic acids in monkey kidney cells infected with poliomyelitis virus, and incubated at 37° C. has previously been reported[1]. To gain more information about the steps involved, an attempt was made to slow down the process leading to virus synthesis. This was accomplished by reducing the temperature of incubation at a time when the first signs of virus activity could be seen in the affected cells, that is[2,3] after 4 hr. incubation at 37° C. Virus release from these cells is known to begin between 5 and 6 hr. after infection[4].

Monolayer cultures of second passage monkey kidney cells were grown on coverslips in Petri dishes containing 10 c.c. of lactalbumin-hydrolysate supplemented with 0·1 per cent yeast extract and 10 per cent horse serum in an atmosphere of 5 per cent carbon dioxide in air at 37° C. After two saline washings they were massively infected with either Type I Mahoney strain or Type II MEF_1 strain in a concentration sufficient to infect almost all the cells in ½ hr. Following an adsorption time of ½ hr., fresh medium was added, and after 4 hr. of incubation at 37° C., the cultures were further incubated at either 30° C. or 23° C. Controls were kept at 37° C. After various times coverslips were removed and fixed in methyl alcohol, Carnoy's solution, or 10 per cent formalin buffered to *p*H 7·2. May Grünwald Giemsa, pyronin, and sudan-black were used as stains. In addition living cultures were observed under the phase microscope.

Thread-and rod-like structures were observed in vacuoles in the cytoplasm of infected cells six hours after the incubation temperature was reduced to 30° C. Such structures were never observed in cultures kept at 37° C. The release of rods enclosed in vacuoles is shown in Fig. 1*A*. With more prolonged incubation the number of needle-like structures increased, and they could be seen floating free in the medium, still enclosed in a vacuole. Increased vacuolar activity on the cell surface (bubbling) during the second half of the infectious cycle is known to be connected with virus release in cells infected with poliomyelitis[2,3,5].

When the incubation temperature was lowered to 23° C. release of rods and slabs from the infected cells

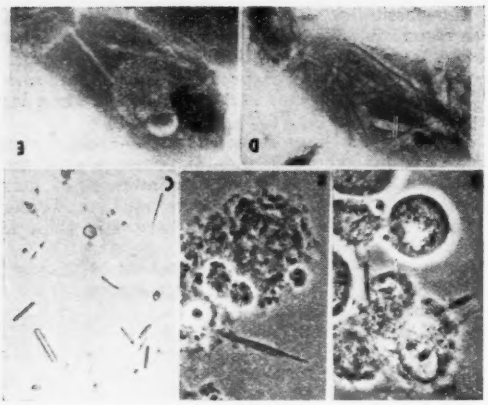

Fig. 1. (*A*) and (*B*), polio-infected living monkey kidney cells (phase microscope, × 625); (*A*), incubated for 4 hr. at 37° C. followed by 6 hr. at 30° C.; (*B*), incubated for 4 hr. at 37° C. followed by 20 hr. at 23° C.; (*C*), crystalline needles collected by centrifugation from the nutrient medium (× 450); (*D*) and (*E*), formalin-fixed and May Grünwald Giemsa-stained polio-infected cells, incubated for 4 hr. at 37° C. followed by 12 hr. at 23° C. (× 700)

occurred later than in the previous series. Rigid angular hyaline structures surrounding a centrally located dark rod protruded from the infected cells (Fig. 1*B*). When viewed under the phase contrast microscope, these structures were seen to rotate slightly due to their loose anchorage to the cell body and light weight (free swimming rods in the medium exhibited a rapid Brownian movement). In a certain position they appeared as flattened slabs, narrow at the base and gradually becoming wider at the distal end.

After centrifugation at 3,000 r.p.m., rods (Fig. 1 *C*) could be recovered from the upper surface of the supernatant fluid. They showed birefringence under polarized light. Experiments to isolate a greater quantity of the needles and to test their infectivity are in progress.

In stained and fixed preparations different stages of needle formation could be observed. Fig. *D* 1 shows a formalin-fixed and May-Grünwald stained cell, 16 hr. after infection (12 hr. at 23° C.). This cell is in an advanced stage of the infectious cycle judging by the collapsed, highly pycnotic nucleus. In the cytoplasm many basophilic threads surrounded by an unstained layer can be seen. In addition, short differently shaped angular structures were frequently seen in the cell cytoplasm in these cultures. They consisted of a rich deposit of a basophilic material surrounded by a layer of an unstained material. When treated with 0·075 per cent ribonuclease in buffered saline for one hour at 37° C., the basophilic core of the rods, as well as of the other basophilic cytoplasmic inclusions mentioned above, disappeared. This would suggest the presence of material containing ribonucleic acid.

The low temperature of incubation also affects the development of the eosinophilic perinuclear cytoplasmic inclusion described for polio-infected cells[3,5]. In infected monkey kidney cell cultures incubated at 37° C., these structures appear regularly about five hours after infection; at the low temperature they are less frequent. In stained preparations these bodies can be seen to contain very fine, faintly greyish stained granules, surrounded by hyaline haloes, a structural detail not seen at the normal incubation temperature. Usually the rods do not penetrate this body, if present in the same cell, and the impression was gained that the basophilic core, if formed at all, was only faintly stained (Fig. 1*E*).

Previous investigations on the cytopathogenic effect of the polio virus on cell cultures incubated at low temperatures have not shown any of the changes described here[5–7]. These changes are believed to appear under the present experimental conditions when the temperature is lowered after an initial incubation of not less than 4 hr. at 37° C.

A full report of this work will be published elsewhere.

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BIOLOGY

Effect of Photoperiod on the Gonadal Cycle in an Equatorial Bird, *Quelea quelea*

Rowan's[1] discovery that increasing day-lengths in winter induce reproductive activity and migratory behaviour has been amply confirmed for many species and has contributed greatly to our understanding of the timing of these events in birds of the north temperate zone[2]. There still remain, however, the problems of regulation of migration in species that winter in the tropics and south temperate zone and regulation of breeding in species that are permanent residents in the tropics. The great dissimilarities in day-length conditions in these zones, and particularly the relative constancy of day-length in the equatorial region, have raised considerable doubt about a possible regulatory function of day-length in equatorial and trans-equatorial migrants and in tropical species.

In view of the importance of day-length in north temperate zone species, it seemed reasonable to assume that day-length was important in these other species, but perhaps was operating in a different way. To learn more about the relation between day-length and the reproductive and migratory responses, an extensive series of experiments was performed using first north temperate zone species. These studies showed that the time of breeding and migration is regulated in two separate phases—a preparatory phase, which occurs in the fall, and a subsequent progressive phase which occurs in late fall, winter, and spring. Both phases are regulated by day-length, short days, or more specifically long daily periods of darkness, being required for completion of the preparatory phase, and the rate of response and its duration in the progressive phase being a function of the photo-

period[3]. These conclusions, although derived wholly from studies of north temperate zone species, appeared to be applicable to equatorial and trans-equatorial migrants as well as to resident tropical birds.

Through the interest and generous co-operation of Dr. A. J. Marshall and Mr. H. J. de S. Disney we received approximately 200 red-billed weavers (*Quelea quelea*) from Dodoma, Tanganyika Territory (6° S. latitude) on October 27, 1958, and our experimental programme began, therefore, with the problem of the timing of the breeding cycle in this resident tropical bird. Marshall and Disney have experimented with this same species and have reported on its breeding biology[4]. They found that the males were susceptible to photo-stimulation, but they believe that it is the environmental conditions arising after rainfall, operating in conjunction with the internal sexual rhythm, that constitute the critical factors regulating the time of reproduction. The experiment reported here was designed to explore the relation between the daily photoperiod and the phases of the internal sexual rhythm. Observations were also made on body weight, subcutaneous and intraperitoneal fat depots, and moult; these data will be reported at a later date.

Shortly after the birds arrived in Evanston they were placed in an aviary with a constant daily photoperiod of 12·75 hours, the approximate mean photoperiod (including civil twilight) at 6° S. latitude. On November 21, three groups of birds, 24 in each, were formed and treated with the following constant daily photoperiods: 16·75 hours, 12·75 hours, and 8·0 hours. At this time of the year the birds were approaching their normal breeding season which occurs from December to June in East Africa. During the period of observation being reported on here, November 21 to April 9, the birds were weighed approximately every two weeks and observations made on reproductive activity using methods already described[5]. In addition, some birds were autopsied at critical intervals to determine weights of reproductive organs and for histological study of the gonads.

The weights of the testes shown in Fig. 1 indicate that maximal gonadal activity was attained in all groups by January 31. It is not known precisely when gonadal recrudescence began, but change in bill colour of the female, which is under the control of œstrogen, and the growth of nuptial plumage in the male, which is under the control of luteinizing hormone of the pituitary[6], began in December. The photoperiod apparently did not alter the growth of the testes, but as shown in Fig. 1 it did have a marked effect on the duration of testicular activity. The 8-hour group began to show regression of reproductive activity in late February and March, and by April 9 the testes were at minimal size. Loss of body weight and fat deposits and initiation of annual molt also occurred concomitantly in the 8-hour group. In sharp contrast, the birds in the other groups continued their reproductive activity, and as late as April 17 none of the individuals had begun to molt. Few females were autopsied in each of the groups, but the gonadal data obtained agree essentially with the pattern seen in the males, with the possible exception that ovarian activity was not as great in the 8-hour group; observations on bill colour were in agreement.

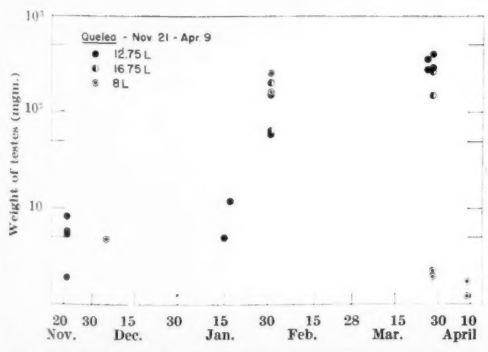

Fig. 1. Weight of testes in the three experimental groups, plotted semi-logarithmically. Each point represents the combined weight of the testes in each individual

The gonadal response in the present experiment differed markedly from the response of *Junco hyemalis*, a temperate zone species, under similar experimental conditions[3], but similar relationships between photoperiod and the phases of the gonadal cycle are not to be expected. If day-length conditions govern the duration of gonadal activity in the tropics, as they appear to do in the north temperate latitudes, then day-length would play a primary role in determining the time of breeding in the tropics. Such a role would be critical if a preparatory phase existed, and if the duration of the preparatory phase was also a function of day-length. Further study of these same three groups of birds, and additional studies which are in progress, are expected to yield information bearing on the preparatory phase of the gonadal cycle. Data on other aspects of the present study and the results of the studies on the preparatory phase will be published later.

We wish to thank Dr. A. J. Marshall and Mr. H. J. de S. Disney for making it possible for us to obtain the *Quelea*. We are indebted to Dr. Marshall also for stimulating and valuable discussion of the general problem. The research was supported by the National Science Foundation and Northwestern University.

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Duration of the Regeneration Period of the Internal Reproductive Rhythm in a Xerophilous Equatorial Bird, *Quelea quelea*

THERE exist two widely divergent views on the external means by which the breeding seasons of equatorial birds are regulated. One view is that, although the sexual cycle of at least one such species (*Quelea quelea*) retains an innate susceptibility to photo-stimulation[1] it is other and various environmental effects (often those arising after rainfall) that operate in conjunction with the internal rhythm and so stimulate breeding irrespective of the cycle of the Sun[2]. The opposing argument[3] is that the sexual cycle is governed by photoperiodicity, as is the case in many species living in higher latitudes.

In the spring-breeding temperate zone species so far investigated there intervenes at the end of the breeding season a period of refractoriness or sexual negativity during which the males do not respond to artificial photo-stimulation[4]. This is more correctly a period of regeneration (or preparation[3]) that lasts for a period of four or five months[5].

It was considered of interest to determine whether this temporary period of enforced sterility intervenes also in the reproductive rhythm of xerophilous equatorial species for which it is beneficial that reproduction should occur at any time of the year when rain falls and produces conditions propitious for breeding and the survival of young[2,6].

Q. quelea is a nomadic weaver-finch which in East Africa oscillates back and forth across the equator according to the available food supply. Thirteen Tanganyika males in full nuptial plumage were taken from our London colony which is kept under the equatorial day-length of 12 hr. These were laparotomized on February 12. The gonads measured 10 × 5 mm. and were approximately at the peak of breeding condition. One bird was killed and found to be in full spermatogenesis.

The 12 survivors were now subjected to a daily photoperiod of 17 hours[1]. On March 15 they were still in full nuptial plumage and were again laparotomized. The testes, however, now measured a mere 1·5 × 1 mm. Two birds were killed and their seminiferous tubules were in the fully metamorphosed condition that indicates the onset of 'refractoriness'[7]. Of the ten surviving birds, half were now transferred to an equatorial day-length of 12 hours, and half were retained under the existing régime.

On March 20 unilateral castrations revealed that in both groups the gonads of a minority were beginning to develop. The experiment was terminated on April 6 when the survivors were killed for examination. In all but two birds primary or secondary spermatocytes had arisen.

The results indicate that in this xerophilous equatorial species a post-nuptial period of sexual regeneration exists, but that it is relatively brief. Thus, after each breeding season the males, at least, progress with little delay into a condition whereby they can take advantage of propitious breeding conditions whenever these arise in the arid environment to which they, and especially their reproductive processes, are adapted.

Details of this and a collateral experiment will be published elsewhere.

We are grateful to the the Nuffield Foundation support for this work.

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Force-Feeding Fish under Experimental Conditions

It is a common experience in aquarium management that fish in captivity vary greatly in the readiness with which they will accept food, and there is no doubt that the refractory species feed less readily after surgical operation. Surgery also lowers the general resistance of the subject, and the effects of inanition are more drastic. Over the past six years we have carried out thyroidectomies, and partial and total hypophysectomies on several hundred dogfish, and in the initial experiments, in which food was merely placed in the aquarium tanks, we experienced poor post-operative survival, usually of the order of 4 weeks and in no case exceeding 8 weeks. A review of the relevant literature showed this to be the experience of all previous workers with the exception of Matty[1], who thyroidectomized *Scyliorhinus caniculus* and reported that all the operated fish began to feed on strips of fresh squid muscle within a few days of the operation. He obtained post-operative survival times of "at least seventy-two days".

It is not known whether hypophysectomized fish would accept squid muscle, or whether such a diet would give an acceptable survival time, since squid is not usually available in this Laboratory. But even if it was found to be satisfactory from these points of view, the difficulty still remains that when food is offered *ad lib.* it is impossible to ensure that operated fish and their sham-operated controls take the same amount.

This is a consideration of extreme importance in experimental work involving hypophysectomy, since inanition itself can produce many of the effects which result from this operation. We have, therefore, investigated the possibility of force-feeding using minced herring, which is available throughout the year and has a consistency which is easily handled by the feeding devices in use. Two satisfactory methods have been evolved, and these have enabled us to keep operated dogfish (*S. caniculus*) alive and in good condition for more than two years.

Method 1. This method is based on that of Vivien[2] who inserted whelk, *Patella* and crab into the stomachs of dogfish (*S. caniculus*) by means of a glass tube fitted with a plunger, and as a result obtained survival of a few fish for almost a year. We use commercially available plastic vaginal applicators (manufactured by Ortho Plastics Ltd.), which are cylindrical syringes some 14 cm. long with a uniform bore 1 cm. in diameter. They carry a piston by means of which they can be completely emptied. The applicators are filled from a confectioners' icing syringe, the nozzle of which has been adapted for the purpose by enlarging its aperture to a diameter of 1 cm.

After charging the icing syringe by hand, the nozzle is applied to the mouth of the applicator and a jet of herring is injected into the barrel, completely filling it and forcing the piston out against its stop. Each applicator holds 6 gm. minced herring, and the contents of six applicators adequately fill the stomach of an adult dogfish of some 800 gm. Feeding is carried out by placing the fish on its back, reflecting the lower jaw with a protected finger or stout forceps and gently easing the tip of the applicator through the cardiac sphincter into the stomach. Slight difficulty is usually experienced in introducing the first applicator, and care is necessary to prevent abrasion of the œsophageal epithelium.

Method 2. The above method is adequate if the number of fish to be fed is not more than thirty. However, larger numbers than this are frequently involved and it has proved necessary to mechanize the feeding procedure. A machine has been devised for this purpose, the constructional details and dimensions of which are shown in Fig. 1. It is essentially a modified sausage-making machine consisting of a cylinder mounted on a vertical rod with a clamp at its base. The cylinder can be fixed at any point along the rod to suit the convenience of the operator. It holds 230 gm. herring which is expressed by means of a steel cup piston operated by a rod to which a handle is rigidly attached. The piston moves through the cylinder at a uniform rate when the handle is turned, since the rod is threaded (quick-lead thread) into the bayonet cap fitting of the cylinder. The metal adaptor at the base of the nozzle carries a flexible rubber tube, 16 cm. × 1·3 cm., into which is inserted a rigid polythene tube, 13 cm. × 1 cm.

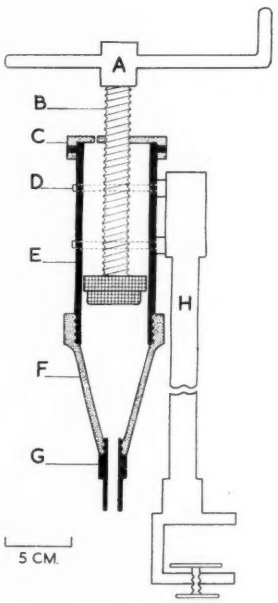

Fig. 1. Mechanical feeding device. *A*, handle to which is attached threaded rod *B*, and metal plunger; *C*, end cap with bayonet fitting and air hole; *D*, metal band for attachment of cylinder *E* to vertical supporting rod *H*; *F*, nozzle threaded on to lower end of cylinder; *G*, adaptor to take rubber and plastic tubes; *H*, vertical rod and clamp: overall length 60 cm.

Feeding can be carried out by a single operator who, holding the fish vertically, opens its mouth to allow entry of the polythene tube and raises the fish gently until the tube enters the stomach; he then turns the handle the requisite number of times (each complete turn ejects 8 gm. herring) until the desired amount has been delivered. In this way fifty dogfish can be fed in one hour. Each receives the same amount of food; moreover, satisfactory growth-rates have been recorded, and females fed in this way lay normal looking eggs at regular intervals throughout the egg-laying season.

No doubt these methods of force-feeding can be used for a considerable variety of fish, and Mr. A. Barr has recently modified the plastic applicator for use with plaice (*Pleuronectes platessa*). This has involved reducing the diameter of the aperture by adding a short piece of thick walled rubber tubing, external diameter 0·9 cm., which is long enough to enter the stomach of the plaice. The fish are fed twice a week, each receiving 6–30 gm. herring per meal, depending on their size. As a result, several fish are still alive eight months after total hypophysectomy.

We are grateful to Mr. G. Rae-Arnot who made the mechanical feeding device and also gave us advice when we were designing it.

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Estimating Earthworm Populations by Using Formalin

While investigating the removal of leaves by earthworms from the soil surface of some apple orchards at Wisbech (Cambs.) an estimate of the population of *Lumbricus terrestris* was needed because it seemed to be the only species present that pulled apple leaves into its burrows. *L. terrestris* burrows deeply in the light, well-drained soil of the Wisbech area, and a population estimate made by removing and hand-sorting soil samples was impracticable because of the depth of the burrows into which the worms retreat when soil samples are removed. The permanganate method used by Evans and Guild[1] has been shown by Svendsen[2] greatly to under-estimate the population and my tests at Wisbech confirmed this.

In an arable orchard undisturbed during the winter, I found that, when dilute formalin was used to expel the worms, a population estimate was obtained which corresponded very closely with that made by counting the number of burrows into which leaves had been drawn. For comparison, population estimates were made in April 1959 by the formalin, potassium permanganate and hand-sorting methods in the arable orchard and in an orchard under grass.

Formalin method. 25 ml. of 40 per cent formalin was added to 1 gall. of water and the solution applied to a quadrat of 4 sq. ft. A second application was given when the first ceased to bring worms to the surface, usually after about 20 min.

Permanganate method. As in the formalin method, using a solution of 7 gm. potassium permanganate in 1 gallon of water for each application.

Hand-sorting. Soil samples 8½ in. diameter and 8 in. deep were taken with a cylindrical steel sampling tool. It took 2–3 hr. to examine each sample.

In a ½-acre plot in each orchard ten quadrats were treated with formalin and ten with potassium permanganate. Eight soil samples for hand-sorting were taken from the plot in the grass orchard and sixteen from the plot in the arable orchard where earthworms were fewer. The worms were collected into 5 per cent formalin, identified and weighed. The weight after preservation in 5 per cent formalin is about 25 per

Table 1. Average Number and Weight of Worms and Number of Burrows per Quadrat of 4 sq. ft.

Species	Arable orchard			Grass orchard		
	Formalin	Potassium permanganate	Hand-sorting*	Formalin	Potassium permanganate	Hand-sorting*
Lumbricus terrestris adult	6·4	1·4	0	7·5	3·1	0
L. terrestris immature > 5 cm.	14·0	3·8	3·1	17·7	5·1	7·4
L. terrestris immature < 5 cm.	18·6	13·1	10·6	31·2	13·5	14·0
L. castaneus	3·4	3·7	0·6	18·6	1·7	32·7
Allolobophora chlorotica	9·5	6·9	15·6	19·3	6·5	31·8
A. caliginosa	0·6	0	3·8	52·6	41·4	118·0
A. terrestris	0	0	0	3·1	1·4	7·7
Eisenia rosea	2·4	0·3	7·5	11·3	9·4	55·2
Octolasium cyaneum	4·8	3·2	6·3	3·0	1·4	8·2
Undetermined	0	0·1	0	0·8	4	5·0
Total	59·7	32·5	47·5	165·1	83·9	280·0
Weight of *L. terrestris* (gms.)	53·5	12·7	5·0	68·7	19·1	4·8
Weight of other species (gms.)	3·0	3·0	3·4	19·5	11·5	37·8
Total weight	56·5	15·7	8·4	88·2	30·6	42·6
Burrows of *L. terrestris*		18·5				

* These estimates include worms of which only pieces were found, and weighed

cent less than the fresh weight. The results in Table 1 are expressed as the average number and weight of worms per quadrat of 4 sq. ft.

The *L. terrestris* could be divided readily into three groups; mature specimens, weighing 2·5–8·0 gm.; medium to large immature specimens, 5 cm. or more in length when preserved and weighing 0·7 gm. or more; and small immature specimens usually 2–3 cm. long and weighing 0·1–0·25 gm. The last group, probably hatched from egg capsules since autumn, 1958, seemed to be too small to pull leaves into burrows and the older, larger worms of the first to groups were undoubtedly those which had formed burrows and were able to pull leaves into them. The estimate of 20·4 such worms per quadrat obtained by the formalin method corresponds closely with that of 18·5 per quadrat obtained by counting burrows visible on the surface. The permanganate method gave a much lower estimate and the soil samples which were hand-sorted were clearly too shallow to permit an accurate estimate.

For other species, most of which occur in the top soil, hand-sorting provides an accurate estimate. By comparison, the proportion extracted by formalin varied from 60 per cent for *Allolobophora chlorotica* to 20–32 per cent for *Eisenia rosea* but was consistently higher than the proportion extracted by potassium permanganate.

When the weight lost by preservation in 5 per cent formalin is allowed for, the estimates indicate a fresh weight of *L. terrestris* of 15·3 and 19·6 cwt. per acre in the arable and the grass orchard respectively. The best estimate of the total fresh weight of worms in the grass orchard, made by combining the estimate of *L. terrestris* by the formalin method with that of other species by hand-sorting, is 30·4 cwt. per acre. These are much the highest figures hitherto recorded in Britain and the latter figure is somewhat higher than, but of the same order as, the maximum of 26 cwt. per acre recorded by Waters[3] for productive New Zealand pastures.

Table 2. Total Number of Worms Recovered from 7 Quadrats of 4 sq. ft. for each Treatment

Treatment	Formalin 25 ml./gall.		Formalin 6·3 ml./gall.		Potassium permanganate 7 gm./gall.	
Application	1st	2nd	1st	2nd	1st	2nd
Lumbricus terrestris	3	0	1	2	2	0
L. castaneus	6	1	8	1	3	0
Allolobophora chlorotica	283	22	77	29	41	49
A. caliginosa	140	9	22	15	62	15
A. nocturna	48	2	14	13	28	4
Eisenia rosea	313	20	132	87	54	36
Octolasium cyaneum	1	0	0	0	0	0
Undetermined	2	0	2	0	0	0
Total	796	54	256	147	190	104
Grand Total	850		403		294	

Replicated tests made on a lawn on heavy soil at Rothamsted (Table 2) showed that the concentration of formalin was important in determining the number and proportion of worms expelled at each application. They also showed a much greater difference in the estimates obtained by the formalin and permanganate methods for species other than *L. terrestris* than was observed in the orchards. These tests showed that worms expelled by formalin survived provided they were immediately rinsed in water. Most worms expelled by potassium permanganate die quickly.

Further information about the efficiency of the formalin method and the factors which affect it is being collected.

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Giant Eggs of the Clover Cyst Nematode, *Heterodera trifolii* Goffart, 1932

Impregnation of the females of the clover cyst nematode, *Heterodera trifolii* Goffart, 1932, by males of the sugar-beet nematode, *H. schachtii* Schmidt, 1871, was reported in 1958[1]. The activity of the sugar-beet nematode sperm in the reproductive tract of the clover cyst nematode and the production of giant eggs is now reported.

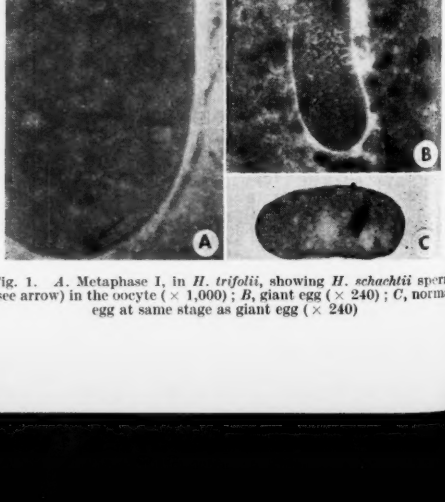

Fig. 1. *A*. Metaphase I, in *H. trifolii*, showing *H. schachtii* sperm (see arrow) in the oocyte (× 1,000); *B*, giant egg (× 240); *C*, normal egg at same stage as giant egg (× 240)

Mixed cultures of *H. trifolii* and *H. schachtii* were propagated in the manner previously described[1]. Living white gravid females of the clover cyst nematode were removed from the roots of the white Dutch clover plants and prepared for cytological study by the squash technique[2]. *H. schachtii* sperm was found in several oocytes (Fig. 1*A*) and in a few it had proceeded some distance in the cytoplasm of the egg. In the latter the chromatin of the sperm had resolved into discrete chromosomes. However, union of the sperm and egg nuclei was not observed. *H. trifolii* has a diploid number of 27 chromosomes which is not reduced during maturation.

To determine the result of impregnation, white Dutch clover plants were inoculated with live *H. trifolii* females from this population. Giant eggs (Fig. 1*B*) were produced by some females of this second generation. However, both normal and giant eggs were found in these females. Neither the normal (Fig. 1*C*) nor the giant eggs had as yet produced larvæ. Seventeen of the giant eggs averaged 178 μ long (range 140–225 μ) and 45 μ wide (range 40–56 μ). Two meiotic spindles, with many chromosomes, were observed in one giant egg and another contained a large spindle with two sets of chromosomes.

Onions[3] reported the occurrence of a number of giant first-stage larvæ from a single cyst of the golden nematode, *H. rostochiensis* Wollenweber, 1923. Normal larvæ were also present in this cyst. He suggested that either gene mutation or polyploidy were responsible for the formation of these giant larvæ. In the present case it appears that polyploidy has taken place.

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Pattern and Control of Sclereid Formation in the Leaf of *Camellia japonica*

EXCELLENT material for the study of factors controlling cell differentiation may be found in the sclerenchymatous idioblasts present in the leaves of certain higher plants. Outstanding examples of this cell type are the lignified sclereids that occur in leaves of *Camellia japonica* L. During the course of an investigation on sclereid formation in this species I observed the striking distributional pattern of these cells in cleared leaf preparations. In *C. japonica* the sclereids occur predominantly near the lateral margins of the leaf from apex to base and, to a lesser extent, along the length of the mid-vein and scattered throughout the lamina. The distribution of sclereids along a line parallel and close to the leaf margins constitutes a unique pattern, one not hitherto described for any other species.

The sclereids are first recognizable when the leaf is about 4 cm. long and entering the phase of maximum enlargement. In leaves of this size cell divisions do not occur, these having been completed while the leaf was still contained within the bud. Maturing first at the leaf apex, the sclereids develop in basipetal sequence along the mid-vein into the petole. Simultaneously with the maturation of the sclereids along the basal portion of the mid-rib, mature sclerenchymatous elements make their appearance at the bases of the glandular teeth closest to the apex of the leaf. Later sclereids develop basipetally along the lateral margin between the teeth, from the apex to the first tooth, then between the first and second teeth, etc. At the same time mature sclereids appear at the bases of the more basal leaf-teeth, followed by the filling in of marginal sclereids between them, so that a line of sclereids is formed along the entire length of the lateral margins (Fig. 1).

The predominantly marginal position of sclereids in the leaf of *C. japonica* suggests that an important controlling factor in the differentiation of unspecialized parenchymatous elements into sclereid initials is the relative position of the cells to the leaf as a whole. In order to test this idea simple surgical experiments have been performed on young leaves in which the sclereid initials are not yet distinguishable from other parenchyma. These experiments consisted of (*a*) the removal of the marginal area in which sclereids would normally develop, (*b*) the removal of small pieces in the central portion of the blade, and (*c*) simply increasing the extent of the leaf margin by incisions parallel or perpendicular to the long axis of the lamina. Fifty immature leaves were cut in one of the above ways and allowed to grow to mature size on the

Fig. 1. Normal margin (× 33)

Fig. 2. Incised margin (× 33)

plant. In every case mature sclereids were formed along the cut margins (Fig. 2). Sclereids were not formed along the margins incised in mature leaves.

The results of these experiments indicate that the fate of immature cells is in part determined by their relative position in the organ. Cells that would normally have become specialized leaf mesophyll have been made to develop instead into mature sclereids by bringing them into the proximity of a margin during development. Foster[1] has cited the work of Bloch[2], Commoner and Zucker[3] and Bünning[4] on the significance of differential and unequational cell divisions in the determination of cellular specificity in plants with particular reference to idioblasts. It is postulated by Commoner and Zucker[3] that "cellular differentiation may result from the segregation of specific biochemical systems *within* the single parent cell, and that this separation becomes finalized by the laying down of the wall between the two sister cells". This mechanism is apparently not operative in the differentiation of idioblastic sclereids in the leaf of *Camellia japonica*. There is no irreversible sorting out of factors at a cell division. Instead, immature parenchyma possesses a variable potency, as is shown by the fact that cells which would not normally have become sclereids do so when placed in a different position in the organ. Thus these cells do not differentiate autonomously but in relation to their position relative to the total living system.

The idea that cellular differentiation may result from unequal divisions is allied to the cell theory, which

contends that the individual cells are the basic units of organization and govern the varied activities of the organism as a whole. On the other hand, the results reported here provide evidence for the organismal theory. This theory maintains that the entire organism is the primary organizational unit and controls the activities of its constituent cells. The viewpoint of this theory with respect to differentiation has been stated succinctly by Sharp[5]: "Cellular structure is accessory, and cell division is an incident of growth rather than the cause of differentiation." The present work supports the view that the formation and developmental pattern of even highly individualistic cells, such as the idioblastic sclereids of *Camellia japonica*, are under the control of a higher unit than the cells themselves.

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Deoxyribonucleic Acid in Eggs of the Polypodiaceous Ferns

It has previously been reported that in several Polypodiaceous ferns the chromatin of the egg stains progressively less intensely with the Feulgen reagent as the egg matures[1]. In the mature fixed egg a clear area is visible in the centre in a position corresponding with that earlier occupied by the nucleus; but it is impossible to identify any staining with certainty or any sharp boundary to the clear area. The condition of the deoxyribonucleic acid in these eggs raises a problem of considerable genetical and cytochemical interest. A similar condition has been reported in the eggs of some angiosperms[2] and animals[3].

Archegoniate gametophytes of *Pteridium aquilinum* were placed on slopes of Moore's medium, solidified with 1·5 per cent Difco agar and supplemented with 2·4 μc./ml. tritiated thymidine. Two days later they were transferred to unsupplemented slopes and four days later fixed in formol-proprionic-alcohol. Controls showed that in the four days after feeding with thymidine at least one transverse rank of new archegonia had originated and matured. The gametophytes were embedded, sectioned at 5 μ and covered with stripping film. The resulting autoradiographs showed that in the somatic areas surrounding the egg cells in the anterior-most portion of the gametophyte the silver grains were in register with the nuclei, and almost entirely absent from above the cytoplasm. Above the egg the grains were scattered irregularly over its whole section and it has not been possible to detect any correlation between the structures visible in the egg with the phase-contrast microscope and the arrangement of the grains (Fig. 1). When the numbers of grains in a series of adjacent sections were summed, there was no evidence that the total radioactivity incorporated into the egg was any less than that incorporated into neighbouring somatic nuclei, but it was clearly more dispersed.

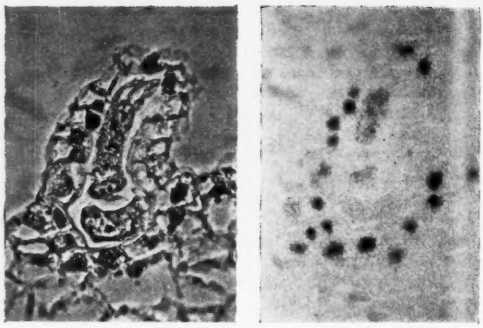

Fig. 1. Left, *Pteridium aquilinum* l.s. mature archegonium showing egg, and the disintegrating ventral and canal nuclei; right, autoradiograph of the same section showing the radioactivity confined to the somatic nuclei, but dispersed throughout the egg (× *c.* 320)

It is generally agreed that thymidine is incorporated specifically into deoxyribonucleic acid. The results of these experiments show that in the mature egg of *Pteridium* the deoxyribonucleic acid is distributed throughout the whole cell. There is, in fact, no recognizable nucleus. A more detailed investigation of the behaviour of the deoxyribonucleic acid during oogenesis and fertilization in *Pteridium* is at present being completed.

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MICROBIOLOGY

Nuclear Structure of Growing Naked Protoplasts of Yeasts

Although many suggestions have been made, the actual nuclear structure of yeast cells is still not known. Contradictions are due to the use of various methods and by different interpretations of artefacts. Only recently have yeast nuclei been studied *in vivo* by phase contrast[1]. Our experiments have shown that the naked protoplasts of yeast and especially the growing protoplats (the so-called plasmatic formations) are, under certain conditions, suitable for the study of the nucleus *in vivo*. The present report covers the results of this research.

Naked protoplasts of *Saccharomyces cerevisiae* were obtained by a method already described[2]. Due to capillary forces a considerable flattening of the protoplasts and plasmatic formations takes place, with the result that the inner structure is easily observable by phase contrast. At the same time a flattening of the nuclei occurs so that division is easily observed. The vitality of the protoplasts is not disturbed as is shown by their growth and regeneration[3].

Under a phase microscope the nucleus appears as a body with a lower refractive index than the cytoplasm (it appears lighter). The shape is usually oval but is often irregular. Sometimes the nuclear membrane is clearly visible. The 'nuclear vacuole' has never been observed. As a rule the nucleus contains a body having a higher refractive index (Figs. 1 and 2). Inside

this small body a small granulum is frequently found. The majority of nuclei (especially in older formations) contain crystalline inclusion. Treatment with reagents such as Feulgen, methyl green–pyronin, and toluidine blue–ribonuclease lead us to believe that the small body inside the nucleus is a nucleolus with a relatively small content of ribonucleic acid. Like the nucleolus in moulds[4] it is intensely coloured by Heidenhain hæmatoxylin while the nucleus proper does not absorb any hæmatoxylin. For we do not known the nature of the refracting granulum inside the nucleolus or of the crystalline inclusions.

Continuous observation, by means of time-lapse cinematography, of the division of nuclei during the growth of protoplasts has shown that the nuclei divide by a simple elongation and subsequent constriction. No structures such as occur during mitosis (spindle, chromosomes, etc.) have been observed. The division of the nucleus is preceded by the division of the nucleolus. The nuclear membrane does not disappear; this is confirmed by observation of ultra-thin sections under the electron microscope[5].

We assume that the structures of nuclei of naked protoplasts and plasmatic formations correspond to the structure of nuclei of normal yeast cells. Thus, according to our observations, yeasts have a nucleus with a low refractive index and a nucleolus with a higher refractive index, and they do not divide during vegetative reproduction by a typical mitosis. The structure of the nucleus of yeast seems to correspond fully to the structure of nuclei of moulds[4,6].

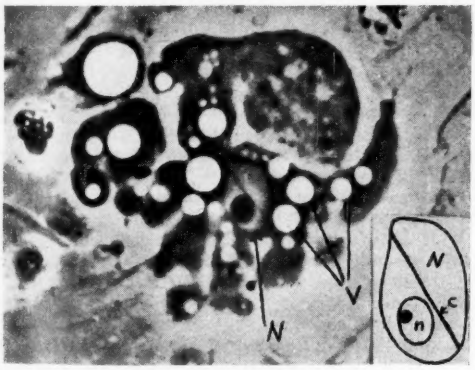

Fig. 1. Flattening of plasmatic formations due to capillary forces. The nucleus is seen as an oval body. Inside the nucleus (*N*) are the nucleolus (*n*) with a refractive granulum and crystalline inclusions (*c*). Numerous round vacuoles in the cytoplasm (*v*). Phase-contrast. (× 660)

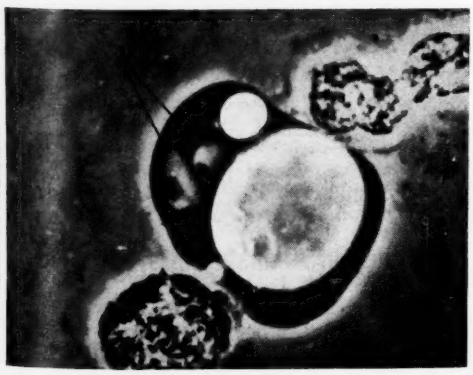

Fig. 2. Binucleate plasmatic formation. Phase-contrast (× 660)

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Staphylocoagulase Inhibition by Pooled Human Gamma-Globulin

Pooled normal human gamma-globulin, alone or in combination with antibiotics, has been found to exert marked therapeutic activity in *Staphylococcus aureus* infections[1–6]. In seeking to understand the mechanism of this protection, it was decided to investigate the effect of gamma-globulin on staphylococcal coagulases, perhaps among the most important factors in the virulence of the organism uncovered to date[7]. Staphylococci form two types of coagulase, bound and free[8,9] which differ in occurrence, mode of action and antigenic patterns but may both be involved in pathogenesis. Evidence has been presented that there are different antigenic varieties of free coagulase associated with the three bacteriophage groups and the two specific types[10], 3*A* and 42*E* but that bound coagulase of the various staphylococcal types may be antigenically homogeneous[9].

In our initial experiment the times required for living, coagulase-positive staphylococci to clot oxalated rabbit blood in the presence and absence of gamma-globulin (27·5 mgm./ml.) were determined. Twenty-four *S. aureus* strains were employed including: 21 different staphylococcal bacteriophage-propagating strains, 1 penicillin-resistant strain (*VCH* 8) of phage type 44*A*/42*B*/52/80/81 isolated from a patient with staphylococcal pneumonia, and 2 additional penicillin-resistant strains (Giorgio, *V*A4/47/53/54/75/77 and Rose, 42*B*/52/80/81) selected because of their high degree of virulence for mice. The results are given in Table 1. It can be observed that, with 19 strains, preservative-free pooled normal human gamma-globulin, in the concentration used, inhibited clotting for at least 24 hr.; with the 5 remaining strains, gamma-globulin also inhibited clotting, but for a shorter period of time. When gamma-globulin was tested at the lower concentration of 5 mgm./ml., blood clotting was delayed from 1–24 hr., greater variations appearing among the various strains. Duplicate studies employing 'Bacto' coagulase plasma (Difco) in place of oxalated rabbit blood gave similar clotting inhibition by gamma-globulin.

Table 1. Comparison of times required by 24 different phage types of living coagulase-positive *S. aureus* to clot oxalated rabbit blood in the presence and absence of gamma-globulin (27·5 mgm./ml.)

Phage Group	*S. aureus* No. of types	Hr. required for clotting of: Blood + Saline	Blood + gamma-globulin
I	2	1–3	>24
	1	9	>24
	1	1	4
II	4*	1–4	>24
III	8	1–3	>24
	3†	2–3	4–5
IV	1	2	4
Miscell.	1	1	>24
Miscell.	1 (Rose)	1	>24
III	1 (Giorgio)	1	>24
Miscell.	1 (*VCH* 8)	1	>24

* Includes Strain 3*A*;
† Includes Strain 42*E*.

Table 2. Titres of gamma-globulin required to inhibit free and bound staphylococcal coagulase action

Strain	Minimal amount of gamma globulin inhibiting: Free Coagulase Plasma Coagulation (mgm./ml.)	Bound Coagulase Fibrinogen Clumping (mgm./ml.)
VCH 8	0·43	0·013
Giorgio	0·43	0·013
PS 3A	0·43	0·013–0·054
PS 52	0·11–0·21	0·0067
PS 77	0·11	0·0067

As another facet of the above experiment, it is noteworthy that gamma-globulin also inhibited the fibrinolytic activity of the staphylococci tested. Blood clots induced by these strains failed to dissolve after 24 hr. incubation at 37°C. in either 27·5 or 5 mgm./ml. gamma-globulin, whereas control cultures in the absence of gamma-globulin showed complete clot lysis within this period. Gamma-globulin free of preservative showed no growth inhibition of any of the 24 staphylococcal strains tested.

The pooled normal human gamma-globulin preparation was next investigated for its ability to prevent the coagulation of oxalated rabbit plasma by free coagulase and to prevent the fibrinogen clumping reaction characteristic of bound coagulase[8]. Partially purified free coagulase was prepared by adaptations of published methods[9,10]. Assays of free[10] and bound coagulase[8] were performed by modifications of described procedures. The effects of gamma-globulin on the free and bound coagulases of 5 different *S. aureus* strains were tested. Results are listed in Table 2. The gamma-globulin titre in the case of free coagulase inhibition represented the smallest amount of globulin preventing 3/4 to complete plasma clotting; with bound coagulase, the gamma-globulin titre represented the smallest amount of gamma-globulin inhibiting definite clumping of the staphylococci. From Table 2 it can be seen that the coagulase-inhibitory activity of gamma-globulin varied slightly against different bacterial strains. Considerably less gamma-globulin was required to inhibit bound than free coagulase, under our methods of testing. There was a small but measurable difference (as measured by gamma-globulin inhibition) of the activities of the free and bound coagulases of the 5 different staphylococcal strains tested. With slight variations in anti-coagulase titres, results of the examination of 6 different commercial pooled normal human gamma-globulin preparations (Cutter, Hyland, Lederle, Merck, Parke-Davis and Pitman-Moore) studied showed essentially the same results as those for the preparation reported above. It should be noted that the concentration of pooled gamma-globulin required to inhibit the staphylococcal coagulases *in vitro* was distinctly smaller than that of gamma-globulin present in normal human blood.

Fig. 1. Gamma-globulin inhibition of fibrinogen-serum agar by coagulase-positive *S. aureus* strains *VCH* 8 and Giorgio following 96 hr. incubation. (The secondary fibrinolytic effect in the clouded areas occurs after 48 hr. incubation.)

From Fig. 1, visualization is possible of the effects of adding pooled gamma-globulin to 2 staphylococcal strains growing in a modified fibrinogen serum agar growth medium[11]. Gamma-globulin diffusing from the paper strip has prevented the coagulase-clouding action on the fibrinogen-serum in proportion to the concentration of coagulase diffusing from the bacteria and the gamma-globulin concentration, a function of distance from the paper strip.

These observations indicate that pooled normal human gamma-globulin in small amounts effectively inhibits the free and bound coagulases of living bacteria and of cell-free preparation when tested with rabbit blood *in vitro*. Experiments with the coagulation of human blood have presented essentially parallel findings. Experiments testing the effectiveness of gamma-globulin against the other components of staphylococcal virulence are in progress. Complete details of the methods used in this investigation will be reported elsewhere.

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Inhibition of Actinomycin Synthesis by D-Valine

The actinomycins (I to V)[1] synthesized by *Streptomyces antibioticus* each possess two moles of D-valine per mole of antibiotic[2]; *Streptomyces chrysomallus*, on the other hand, forms a mixture consisting of actinomycins IV (contains 2 moles D-valine), VI

(contains 1 mole D-valine and 1 mole D-alloisoleucine) and VII (contains 2 moles D-alloisoleucine)[2]. During an investigation concerned with the mechanism of synthesis of D-amino acids by *S. antibioticus*, it was observed that the addition of D-valine to a chemically-defined medium[3] in which the organism was growing resulted in a marked inhibition of actinomycin synthesis (Table 1, Fig. 1). The relationship of this event to that seen in penicillin biosynthesis with D-valine[4,5] prompted a further investigation of this phenomenon.

The extent to which antibiotic production is inhibited depends on the concentration of D-valine employed and the length of incubation of the organism. Some variation in the concentration of D-isomer necessary to achieve a given level of inhibition was seen from experiment to experiment; however, the effect was consistently obtained. L-valine, over a wide concentration range, was not inhibitory. In some experiments, particularly when supplied at intervals during a fermentation, it appeared to stimulate actinomycin synthesis suggesting that formation of this amino-acid may be rate limiting for antibiotic production. DL-valine also had a striking effect upon synthesis; the reduction in antibiotic titre observed being due solely to its D-valine content (Table 1). It was further determined that the influence of D-valine was related more closely to antibiotic formation than to growth of *S. antibioticus* since the amount of mycelium produced in the presence of inhibitory concentrations of the D-isomer was found to be similar to that formed in control flasks.

D-valine proved effective when added prior to or during antibiotic production (Fig. 1). For example, the D-isomer (250 μgm./ml.), supplied to the culture after 3, 5, or 7 days incubation checked further synthesis of actinomycin by *S. antibioticus*. The antibiotic titre of the culture medium actually decreased during continued cultivation of the organism, presumably as a result of degradation of the antibiotic previously formed. Destruction of actinomycin by the organism during prolonged cultivation has been observed before (unpublished results).

The D-isomer, supplied before actinomycin synthesis occurred, was generally more effective early in a fermentation; as shown in Fig. 1, the inhibition was overcome to some extent during the late stages of cultivation. It appeared, therefore, that the organism either (*a*) metabolized the D-valine or (*b*) produced a substance which antagonized it. Evidence has been obtained which indicates that the former possibility is the correct one. The disappearance of D- or L-valine from the glutamic acid medium during a fermentation was followed by means of ascending chromatography using the solvent system butanol : acetic acid : water (4:1:5) and Whatman No. 1 filter paper. It was determined that both D- and L-valine (250 μgm./ml. supplied at 30 hr.) were utilized completely; the D-isomer, however, disappearing much more slowly than the L-form (L-valine, 2 days; D-valine, 7 days). It was noted also that within a day following the disappearance of the D-isomer synthesis of actinomycin occurred. The oxidation of D- and L-valine (20 μM) by washed suspensions of *S. antibioticus*, followed in a Warburg apparatus, confirmed the experiments with growing cells. Q_{O_2} (ave. of four determinations) at *p*H 8·1 was: L-valine, 5·1; D-valine, 1·5. Differential permeability could account for these values; however, this has not been investigated as yet.

The valine analogue, α-methyl DL-valine, which inhibits penicillin synthesis[4,5] also prevents actinomycin formation. The effect observed was not as marked as that seen with D-valine, however. After 10 days incubation the reduction in antibiotic titre obtained with α-methyl DL-valine was 13 per cent (250 μgm./ml.), 25 per cent (500 μgm./ml.) and 40 per cent (1,000 μgm./ml.). If L-valine plus analogue were added to the medium, the inhibitory action of the latter compound was virtually abolished; as expected, D-valine did not bring about a reversal of methyl valine inhibition.

Although an equivalent amount of L-valine had no effect on the inhibition due to a given concentration of D-valine (Table 1), the L-form, at high levels, was found to reverse the inhibition to an appreciable extent; for example, with D-valine (200 μgm./ml.) alone, 100 per cent inhibition of synthesis occurred, whereas with D-valine (200 μgm./ml.) plus L-valine (1,000 μgm./ml.), only a 25 per cent reduction in antibiotic titre was seen after 3 days cultivation. Since L-valine is metabolized much more rapidly than its D-form, a single addition of the L-compound to flasks containing D-valine proved effective only in the early period of antibiotic production. However, by supplying the L-isomer at intervals throughout a fermentation, a reversal of D-valine inhibition could be achieved during 7 days production of the antibiotic.

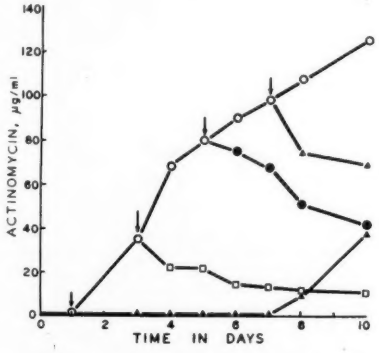

Fig. 1. Inhibition of actinomycin synthesis by D-valine. *Streptomyces antibioticus* was grown in a galactose-glutamic acid-mineral salts medium (ref. 3); D-valine, 250 μgm./ml. (final concentration) was added to flasks at 1, 3, 5, and 7 days after inoculation. The arrow denotes time of addition of D-valine; O, control (no D-valine); ▲, 1 day; □, 3 day; ●, 5 day; △, 7 day.

Table 1. INFLUENCE OF D-, L- AND DL-VALINE ON ACTINOMYCIN SYNTHESIS BY *Streptomyces antibioticus*

Type valine	Concentration added to medium (μgm./ml.)	Actinomycin titre after 10 days incubation (μgm./ml.)	Inhibition or stimulation (%)
None	0	81	0
L-	10	75	−7
D-	10	68	−16
DL-	20	65	−20
L-	50	81	0
D-	50	59	−29
DL-	100	50	−38
L-	250	86	+6
D-	250	31	−62
DL-	500	28	−65
L-	500	83	+2
D-	500	0	−100
DL-	1000	0	−100

Chemically defined medium was composed of (per cent): D-galactose, 1·0; L-glutamic acid, 0·2; disodium hydrogen phosphate, 0·1; crystalline zinc sulphate, 0·0025; crystalline magnesium sulphate, 0·0025; crystalline calcium chloride, 0·0025; crystalline ferric sulphate, 0·0025; distilled water, *p*H 7·2–7·3. When production began (30 hr.) D-, L- or DL-valine was added to flasks of the growing culture.

These results demonstrate that D-valine is an effective inhibitor of actinomycin synthesis. Moreover, the data obtained suggest that L-valine rather than its D-enantiomorph is the precursor of the D-valine present in an actinomycin molecule. The biogenesis of D-valine and the site and nature of the inhibition of actinomycin synthesis occurring in the presence of this amino acid are still to be investigated.

Details of these studies will be described elsewhere.

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ENTOMOLOGY

An Insect attacking *Striga*

Striga is a genus of parasitic plants attacking grain crops throughout the tropical regions of the world. Where monocropping is extensively practised infection can build up to a point where grain yields become negligible. Agricultural techniques such as traperopping[1], soil conditioning and the use of weed-killers can reduce the incidence of the parasite; but in the poorer regions of Asia and Africa where the value of the crop is low but where it remains an essential part of the diet, these techniques are for the most part impracticable.

A study of *Striga* in Nigeria has revealed that in certain areas considerable numbers of the fruits produced by the plant are infected with the larvæ of *Smicronyx* sp. (Coleoptera:Curculionidæ). The eggs of *Smicronyx* are probably laid in the developing fruit or ovary. The larvæ lives within the fruit, feeding on the immature seeds. From preliminary investigations it would seem that the mature larva emerges from the drying fruit and falls to the soil where it pupates. In laboratory culture, larvæ collected in December had all pupated and emerged as adults by the middle of January. The adults have well-developed hind wings, and there seems little doubt that they would fly to new hosts.

Sopubia ramosa is a common alternative host for *Smicronyx* in Nigeria and data from the Commonwealth Institute of Entomology reveals that species of *Smicronyx* have been recorded on *Orobanche* in Cyprus, cotton in the Sudan, *Cuscuta* in India and *Sopubia* in Kenya.

It would therefore seem that *Smicronyx* feeds largely on parasitic plants; but before it could be considered for possible biological control the record on cotton would need careful investigation. The possibility of biological control of *Striga* by *Precis orithya* has been suggested by Agarwala and Naquvi[2], but to our knowledge this has not been investigated further. It has been reported that *Smicronyx* on dodder in Britain lays its eggs in the stem of the plant and the larvæ liva within a gall; but the species under discussion here appears to attack only the fruits.

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PSYCHOLOGY

A Mathematical Model for Perception and a Theoretical Confusion Function

Crossman[1] investigated the time taken to discriminate between stimuli, and produced an empirical expression for the ease of discrimination between two stimuli (their 'discriminability' D) the inverse of which (the 'confusion function') was proportional to the time taken to discriminate. The characteristics of this function were that it depended upon the ratio of the stimulus values concerned, and not upon their absolute magnitudes, and that it tended to 0 and ∞ as this ratio tended to 0 and 1 respectively. The present communication outlines a mathematical model of perception processes, from which a theoretical confusion function was derived.

It is assumed that (*a*) an act of perception is based on a population of values—perhaps, for example, of neurone firing rates—normally distributed about the correct magnitude; that (*b*) the standard deviation of such a population bears a constant ratio (taken as unity for simplicity of computation) to the value of the mean. Thus the distribution for the perception of a stimulus x_i will be:

$$y = \frac{1}{x_i\sqrt{(2\pi)}} \exp\left[\frac{-(x-x_i)^2}{2x_i^2}\right]$$

Clearly, if 2 stimuli, x_1 and x_2, are presented, there will be a finite area ($= A \leqslant 1$) common to both. It is suggested that (*c*) the discriminability is proportional to the area of either not common to both (that is, $D \propto 1-A$) and hence that (*d*) the confusion is proportional to the inverse of this, that is, $\alpha\ 1/(1-A)$.

The curves bounding the distributions connected with x_1 and x_2 ($0 \leqslant x_1 \leqslant x_2$) intersect in the points ($x = p$ and $x = q$, say) given by the roots of:

$$\frac{(x-x_1)^2}{2x_1^2} - \frac{(x-x_2)^2}{2x_2^2} - ln\left(\frac{x_2}{x_1}\right) = 0 \qquad (1)$$

and consequently the discriminability is proportional to:

$$D = 1 - \frac{1}{\sqrt{(2\pi)}}\left[\frac{1}{x_1}\int_{-\infty}^{p} \exp\left\{-\frac{(x-x_1)^2}{2x_1^2}\right\}dx + \frac{1}{x_2}\int_{p}^{q} \exp\left\{-\frac{(x-x_2)^2}{2x_2^2}\right\}dx + \frac{1}{x_1}\int_{q}^{+\infty} \exp\left\{-\frac{(x-x_1)^2}{2x_1^2}\right\}dx\right] \qquad (2)$$

These expressions were solved numerically. The derived confusion values were multiplied by the constant term 1/2·897, for the convenience of giving C = 1 for $x_1/x_2 = 0{\cdot}50$, and are tabulated below.

Table 1. Adjusted Theoretical Confusion Values

x_1 \ x_2	1	2	3	4	5	6	7	8
1	∞	1·00	0·69	0·59	0·54	0·52	0·50	0·48
2		∞	1·64	1·00	0·79	0·69	0·65	0·59
3			∞	2·28	1·32	1·00	0·84	0·75
4				∞	2·92	1·64	1·21	1·00
5					∞	3·57	1·96	1·42
6						∞	4·35	2·28
7							∞	4·86

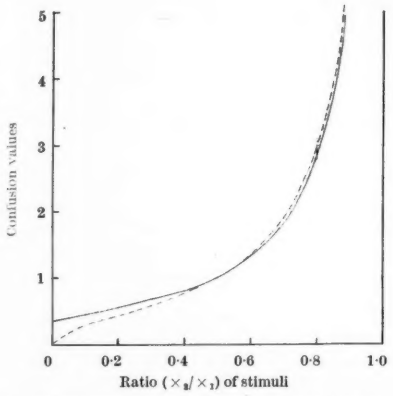

Fig. 1. Curves showing comparison of confusion function values (ref. 1) with theoretical confusion values. ——, theoretical confusion; – – –, confusion function

It will be noted that, as with the empirical function, the value depends on (x_1/x_2), and tends to ∞ when $(x_1/x_2) = 1$. It retains, however, a small finite value (0·34) at $(x_1/x_2) = 0$; but it is with the lower values that experimental results are most unreliable. The generally good agreement can be seen from Fig. 1.

Arguments already published[2] can be used to show that assumptions virtually equivalent to (*a*) and (*b*) above are consistent with the extensive data on sensory thresholds.

In the light of these agreements, this model is tentatively advanced.

I would like to express my thanks to Dr. J. Brown, for his continued encouragement and help.

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MEDICAL STATISTICS

Dose-Response Relationship in Radiation Leukæmia

The nature of the relationship between the dose of radiation, the method of irradiation and the consequent incidence of leukæmia is of considerable theoretical and practical importance. No agreement has, however, yet been reached about the general character of the relationship. This is partly due to the different methods of presentation of the available data, and Dr. D. J. Mewissen's desire to provide a theoretical basis for the use of one particular method[1] is understandable although, in our opinion, mistaken. According to Mewissen, "the development of leukæmia is clearly . . . a quantal response . . . From theoretical statistics we know that quantal effects exhibit a sigmoid dose-response curve. Adequate statistical treatment therefore requires probit analysis". This in our opinion, is not so.

The probit transformation has been found useful in pharmacological and some other investigations, where it is reasonable to suppose that the different responses in different individuals are the result of a distribution of susceptibility or tolerance. It is an empirical fact that this distribution is often a close approximation to log-normal, so that the probit of the quantal response is linearly related to the log-dose. But no particular theoretical model is usually postulated to explain the variability, and other distributions of tolerance give equally good fits. There are, for example, some types of investigation, leading to quantal response data, in which it is reasonable to believe that the responses of different individuals vary because of purely random influences. Examples are provided by experiments involving different doses of some infective agent, like viruses or bacteria[2]. In the simplest model, an individual succumbs if one or more of a certain type of random event takes place. In infective systems, the random event is the successful multiplication of an inoculated organism. In radiation experiments, it might be a particular type of chromosome change. The average number of random events expected for a particular individual is kd, where d is the dose and k is a factor of proportionality. The quantity k measures the susceptibility of a particular individual, and will be constant if all individuals are equally susceptible. In this case, the expected proportion of individuals surviving (that is, not responding) is $\exp(-kd)$. For small doses, that is, for low proportions of responses, $\exp(-kd)$ is effectively equal to $1 - kd$, and the expected proportion of responses is linearly related to d. It can be shown[3] that if k varies from one individual to another the expected proportion of survivors at low doses is effectively $1 - \bar{k}d$, $\bar{k}$ being the mean value of k. In this case, also, the proportion of responses still follows an effectively linear relation to d at low doses.

It seems to us not implausible that in the case of the induction of leukæmia in man the mechanism underlying the dose-response relationship may be of the same random-hit type, rather than of the tolerance distribution type, and it seems reasonable to test this hypothesis by the method appropriate for the conditions under which the disease is observed in man. In the study of patients irradiated for ankylosing spondylitis, which was referred to by Mewissen, the conditions were such that there was no significant immediate mortality (2 out of 13,352 patients may have died as a result of the acute effects of treatment) and the incidence of the observed effect was low (in all, 38 patients developed leukæmia, that is, 0·3 per cent)[4]. Under these conditions, a random-hit mechanism will result in an almost linear relationship between dose and incidence, and the appropriate test of the hypothesis is to make a direct comparison between them. In the spondylitis study, it was assumed, as a first approximation, that the probability of detection of leukæmia was constant in successive years after irradiation, apart from the first, and the incidence of the quantal response was, therefore, regarded as proportional to the incidence per 10,000 person-years of observation.

When, as in many animal experiments, a large proportion of the treated animals die of the acute effects of treatment and a large proportion of the survivors develop the disease, the arithmetical relationship to be deduced from the same hypothesis will be entirely different, and, if incidence is plotted

against log-dose, the resulting curve is likely to be sigmoid in shape. This difference between the conditions under which observations on humans and on experimental animals are usually made may, we believe, be responsible for much of the conflict between the conclusions drawn from the two sets of data.

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A significant contribution is achieved by Drs. Armitage, Court Brown and Doll in evaluating some models postulated in assessing the leukæmogenic potency of radiations. Along this line, it appears relevant to discuss some possible interpretations of a linear dose-response, observed over a limited range of leukæmia incidence, as evidenced in the study of patients irradiated for ankylosing spondilytis. Basic assumptions of some theoretical models will be first examined.

It is an experimental fact that a dose d of radiations will induce leukæmia in a proportion P of susceptible individuals. Clearly, among individuals exposed to the same dose, some respond whereas others do not. Consequently individual susceptibility is postulated. Different theoretical models may be obtained, depending on the assumptions made about the susceptibility. In the random-hit hypothesis referred to by Armitage *et al.*, a factor of proportionality k measures the susceptibility. If k is assumed to be *identical* in each individual, it can be shown that the expected proportion P of individuals remaining unaffected (that is, not developing leukæmia) after irradiation with a dose d is $P=\exp(-kd)$. For small doses associated with low incidence of leukæmia, the exponential is closely approximated by the first term of its expansion $1-kd$. Similarly, if k varies from individual to individual within definite limits, the expected proportion of individuals not developing leukæmia at small doses is $1-\bar{k}d$, $\bar{k}$ being the mean value of k (ref. 1). In both cases the expected proportion of leukæmias will be linearly related to dose *over a limited range*. However, if d or the variation in susceptibility becomes large, the discrepancy between the true law and the exponential increases.

It may be assumed that, in addition to random variations, internal and external conditions result in a widespread distribution of susceptibilities of individuals to radiation leukæmia. The susceptibility k is then defined as a variate with some distribution the density function of which is $f(k)$. It can be shown that the expected proportion of individuals remaining unaffected after irradiation with a dose d, is

$$P=\int_0^1 (\exp(-dk)\ f(k)\ dk \text{ (see ref. 1).}$$

If the susceptibility distribution is assumed to be normal or log-normal with dose, the dose-response relationship will be sigmoid. However, as d or the variation in susceptibility declines, the probability of an unaffected individual becomes more closely described by the exponential law and here also, the expected dose-response curve may be approximated by a linear relationship in a limited range of small doses associated with low response. Thus a simple proportional relationship between dose and incidence in the low range may be an approximation to either one of the exponential or sigmoid theoretical models. Conversely, experimental observation of linearity over a small range does not provide conclusive evidence in favour of the random hit type (exponential) or the susceptibility distribution type (sigmoid). It follows that from human experience both models are plausible as observation of leukæmias was limited to a very low incidence[2].

In animal experiments, data on radiation leukæmia are available over a fairly complete range of incidence. None of those distributed on either sides of a median effective dose is in agreement with an exponential model. On the other hand, probit regression curves have been shown to fit the most complete set of experimental results as yet available[3] in mice. Furthermore, numerous host factors have been[4] identified which influence the leukæmic response in irradiated mice in such a way that the probability of developing the disease may, in specific circumstances, vary from almost 1 to 0, regardless of the dose of radiations absorbed. This experimental fact makes it difficult to consider in theoretical models the susceptibility of mice as a variable submitted to purely random influences. It seems appropriate to assume that k actually has a distribution related to some density function. Indeed, probit analysis suggests that the susceptibility of *C* 57 black mice is distributed log-normally. Thus experimental evidence from animals points to the sigmoid model with a susceptibility distribution.

The considerable theoretical and practical implications of the nature of the dose-response relationship in radiation leukæmia make it desirable to postulate a model which will perhaps fit all available data, from human experience as well as from animal experiments. Obviously, the pathogenesis of human radiation leukæmia may not be identical to murine radioleukæmogenesis. No experimental evidence, however, exists that the respective induction mechanisms are fundamentally different in these species. It seems implausible that the complexity of those mechanisms should be less in humans than in pure inbred mice. Therefore it is doubtful whether susceptibility of man to radiation leukæmia may be properly described by a variable the form of which is simpler than that postulated for mice from experimental evidence. In these circumstances, it is reasonable to assume as a working hypothesis that different responses in different human individuals are the result of a distribution of susceptibility or tolerance. In this sense, probit analysis is the appropriate statistical treatment of a quantal response such as radiation leukæmia.

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